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## ENTOMON

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## Impact of Chloramphenicol Administration on Nutritional Efficiency in a Multibivoltine Race of Silkworm *Bombyx mori*.

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**ABSTRACT:** The popular multi-bivoltine (PM × NB<sub>4</sub>D<sub>2</sub>) silkworm larvae were fed on 25 ppm and 50 ppm concentrations of Chloramphenicol (antibiotic) supplemented mulberry leaf and its effect on food utilization and dietary water intake was studied during fifth instar. Food assimilated, assimilation rate, assimilation efficiency, food converted, conversion rate and conversion efficiencies ( $K_1$  and  $K_2$ ) were significantly higher in the antibiotic treated silkworm batches, though the dry food consumed is on par with the carrier control. Significant decrement in dietary water loss through faeces and transpiration was reflected in higher water absorption efficiency and water retention efficiency in the silkworm larvae fed *ad libitum* with antibiotic supplemented mulberry leaf. Administration of Chloramphenicol resulted in increased conversion of food in to shell content, indicating its beneficial results of higher silk synthesis. The factors responsible for decreased food assimilation, conversion, larval biomass, cocoon weight, pupation rate and shell weight in the Chloramphenicol treated silkworms are discussed. © 2001 Association for Advancement of Entomology

**KEYWORDS:** bio-energetics, *Bombyx mori*, chloramphenicol, silkworm.

### INTRODUCTION

The silkworm *Bombyx mori* feeds exclusively on the leaves of mulberry plants and thus *Morus alba* forms the basic food for silkworms. The growth and development of silkworms and their economic characters are influenced to a great extent by the nutritional content of mulberry leaf (Aftab Ahamed, 1994; Shivakumar, 1995). The quality of mulberry leaves as food for silkworms greatly affects the cocoon crop performance (Shivakumar, 1995). Food ingestion, digestibility and growth in the larval stage are interrelated and the rate of digestion in silkworm increases with the advancement of instar, which is highest, about 65% in the fifth instar (Ueda, 1982).

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The growth promoting effect of antibiotics has been demonstrated earlier in silkworms (Shyamala *et al.*, 1960, 1976; Verma and Atwal, 1963; Verma and Kushwaha, 1971; Radha *et al.*, 1981; Barman and Pasha, 1985). The mechanism of action of antibiotics in biomass accumulation is still incompletely understood. There is controversy as to whether the antibiotic acts entirely through its antibacterial property or by favorably affecting the physiology and metabolism of the host organism itself. The antibiotics may affect the metabolism by an increase in the feed efficiency or by the activation of enzymes or through hormones, which control and regulate growth (Shyamala *et al.*, 1960; Verma and Kushwaha, 1971). Shyamala *et al.* (1976) have also reported that the growth promoting activity exerted by Chloromycetin and Aureomycin in the larvae of silkworm *Bombyx mori*. It has also been shown that an increase in silk proteins occurs when an extra nitrogen source is provided along with the antibiotic to the silkworm diet (Murthy and Sreenivasaya, 1953; Govindan *et al.*, 1990). Further, more evidences have been provided on the activation of glutamic, aspartic acid transaminase in the tissues of the silkworm on chloromycetin supplementation (Shyamala *et al.*, 1960). In the present study an effort has been made to examine the impact of feeding mulberry leaves supplemented with Chloramphenicol (antibiotic) during fifth instar on food and dietary water utilization and cocoon conversion efficiencies in a popular multi-bivoltine silkworm hybrid (PM × NB<sub>4</sub>D<sub>2</sub>).

#### MATERIALS AND METHODS

The silkworm larvae of multi-bivoltine hybrid (PM × NB<sub>4</sub>D<sub>2</sub>) were reared upto fourth moult in recommended environmental conditions (Krishnaswami, 1978). The freshly moulted fifth instar larvae were grouped into three batches, each batch with three replications of 100 larvae each and were reared at the temperature of  $25 \pm 1^\circ\text{C}$  and a relative humidity of  $75 \pm 5\%$ . The batch-1 larvae are considered as carrier control where, the larvae were fed with normal mulberry leaf sprayed with distilled water. However, the batch-2 and batch-3 are experimental batches where, the larvae fed with two different concentrations of antibiotic chloramphenicol (E. Merek) viz., 25 ppm (batch-2) and 50 ppm (batch-3) supplemented mulberry leaf. The treatment was initiated on the first day of fifth instar by spraying chloramphenicol solution at varying concentrations separately. The silkworm larvae of the above 3 batches were fed *ad libitum* on mulberry leaves four times a day. Before feeding, the fresh leaves of mulberry were cut into two halves, one portion was used to find out the initial water content (Delvi and Pandian, 1972) and the other half was weighed and sprayed with known volume of chloramphenicol by using an atomizer. The quantity of leaf offered to all the batches was similar. The left over leaves and faecal matter were dried in a hot air oven at  $80^\circ\text{C}$  to get constant weights and the values were recorded. Similarly, the sample larvae from each group were dried to determine the dry weights. However, the initial and final wet weight of the larvae were recorded from all the three batches to determine the larval growth. Daily food and dietary water intake and their utilization were tabulated by following the standard gravimetric method described by Waldbauer (1968). The rates and efficiencies of food and water utilization were

computed according to the method of Delvi (1983). The rates and efficiencies of food utilization were estimated using IBP equation ( $I = B + M + F$ ) where,  $I$  = Food ingested,  $B$  = Biomass gained,  $M$  = Food metabolized ( $I - F$ ) -  $B$ ,  $F$  = Faeces produced,  $I - F$  = Food digested and assimilated (Waldbauer, 1968). The feeding, assimilation, conversion and metabolic rates were calculated for mid-body weight ( $b$ ) and duration of instar ( $T$ ) for food consumed ( $I$ ), assimilated ( $I - F$ ), food converted ( $B$ ) and food oxidized ( $M$ ). Food assimilation efficiency was calculated by digested food for food consumed and conversion efficiency was calculated both for ingested ( $K_1$ ) and digested ( $K_2$ ) by taking into account of gained biomass (Delvi, 1983).

The dietary water intake and utilization rates were calculated based on wet food intake and other parameters were carried out by similar method described above (Delvi, 1983). The larval biomass, cocoon, pupa and shell weights were recorded and conversion efficiencies of these parameters were calculated for wet food ingested (Aftab Ahamed *et al.*, 1999).

For example

$$\text{Larval biomass conversion efficiency} = \frac{\text{Maximum larval weight}}{\text{Wet food ingested}} \times 100.$$

The rates and efficiencies of food and water utilization were statistically analyzed by adopting ANOVA test and LSD method for mean separation. The mean values followed by different alphabets in all the tables are significantly different with each other by  $P < 0.05$ , such assessment is carried out by applying 'Duncan Test' (Duncan, 1955). The units of intakes are expressed in mg/worm/5th instar, the rates are in mg/mg live weight of insect/day and the efficiencies are in percentages.

## RESULTS

The fifth instar larval duration was on par with control in both Chloramphenicol supplemented batches (25 ppm and 50 ppm) where, the larvae of these three batches took 7 days and 18 hours to complete their fifth instar period. Similarly, the amount of dry food consumed, food oxidized and metabolic rate were also on par with control in both Chloramphenicol supplemented batches (Table 1). However, the amount of faeces produced and feeding rate in Chloramphenicol treated batches were decreased significantly ( $P < 0.01$ ) when compared with the control whereas, in 25 ppm treated batch though, the feeding rate was lower than the control it was not statistically significant (Table 1). A significant increment ( $P < 0.01$ ) in the amount of food assimilated, assimilation rate, assimilation efficiency food converted, conversion rate, gross and net conversion efficiencies ( $K_1$  and  $K_2$ ) were noticed in both 25 ppm and 50 ppm Chloramphenicol treated batches when compared to control batch (Table 1).

The dietary water intake and utilization parameters exhibited a significant ( $P < 0.01$ ) increment in water absorption efficiency, water retained in the body and retention efficiency in the Chloramphenicol treated batches whereas, in 25 ppm treated batch the water retention efficiency was not significant when compared to control batch (Table 2). However, a significant ( $P < 0.01$ ) decrement in water loss through

TABLE 1. Impact of Chloramphenicol on food intake and utilization in a multi-bivoltine hybrid silkworm race (CB) during 5th instar

Parameter	Control	Chloramphenicol		CD	
		25 ppm	50 ppm	5%	1%
Larval duration (D:H)	7:18	7:18	7:18	NS	—
Dry food consumed	2980.6	2978.9	2940.5	NS	—
Faecal matter	1651.0 <sup>a</sup>	1344.0 <sup>b</sup>	1300.0 <sup>c</sup>	41.6	61.7
Food assimilated	1329.6 <sup>a</sup>	1634.9 <sup>b</sup>	1640.5 <sup>b</sup>	111.3	168.6
Food converted	501.4 <sup>a</sup>	681.2 <sup>b</sup>	699.0 <sup>c</sup>	12.8	19.4
Food metabolized	828.2	953.8	941.5	NS	—
Feeding rate	0.1753 <sup>a</sup>	0.1664 <sup>a</sup>	0.1520 <sup>b</sup>	0.012	0.018
Assimilation rate	0.0781 <sup>a</sup>	0.0902 <sup>b</sup>	0.0848 <sup>b</sup>	0.006	0.009
Conversion rate	0.0295 <sup>a</sup>	0.0376 <sup>b</sup>	0.0361 <sup>b</sup>	0.002	0.003
Metabolic rate	0.0486	0.0526	0.0487	NS	—
Gross conversion efficiency ( $K_1$ )	16.8 <sup>a</sup>	22.9 <sup>b</sup>	23.8 <sup>c</sup>	0.783	1.2
Net conversion efficiency ( $K_2$ )	37.8 <sup>a</sup>	41.7 <sup>b</sup>	42.6 <sup>b</sup>	2.8	4.3
Assimilation efficiency	44.6 <sup>a</sup>	54.9 <sup>b</sup>	55.8 <sup>b</sup>	2.3	3.6

Mean values: the values of intakes are expressed in Mg/worm/5th instar, the rates are in mg/mg live weight of insect/day and the efficiencies are in percentage. Means in a row (for one parameter) followed by different alphabates (*a*, *b* and *c*) are significantly different  $P < 0.05$  (LSD). NS - Non-significant.

faeces and the rate of water loss through faeces was noticed in both 25 ppm and 50 ppm Chloramphenicol treated silkworm batches (Table 2). Similarly a significant ( $P < 0.01$ ) decrement in water intake rate, water absorption rate and rate of water loss through transpiration was observed in 50 ppm treated batch whereas, in 25 ppm treated batch they were not significant when compared to control values (Table 2). The water loss through transpiration was also significantly ( $P < 0.05$ ) lower in 50 ppm treated batch than that of control batch whereas, in 25 ppm batch it was not significant (Table 2). However, the amount of water intake and absorbed were more or less similar in 25 ppm and 50 ppm Chloramphenicol treated batches when compared to control (Table 2).

Further, the wet food consumed/worm/5th instar was not significantly different in the Chloramphenicol treated silkworm batches (2 and 3) and it was on par with the control batch (Table 3). However, a significant ( $P < 0.01$ ) increment in the pupation rate, cocoon shell ratio (CSR), weights of larvae, pupa, cocoon, shell and their conversion efficiencies were noticed in both 25 ppm and 50 ppm Chloramphenicol treated batches when compared with control batch (Table 3). However, in 25 ppm Chloramphenicol treated batch, the cocoon shell ratio (CSR) and shell conversion efficiency were similar to the control values (Table 3). There was significant increase in larval biomass was observed in both the Chloramphenicol treated batches when

TABLE 2. Impact of chloramphenicol on dietary water intake and their utilization in a multi-bivoltine hybrid silkworm race (CB) during 5th instar

Parameter	Control	Chloramphenicol		CD	
		25 ppm	50 ppm	5%	1%
Water intake	7588.7	7698.3	7598.8	NS	—
Water loss through faeces	990.2 <sup>a</sup>	886.4 <sup>b</sup>	913.2 <sup>b</sup>	45.4	68.7
Water absorbed	6598.5	6811.8	6685.6	NS	—
Water retained in the body	1933.6 <sup>a</sup>	2160.5 <sup>ab</sup>	2377.7 <sup>b</sup>	235.1	356.1
Water loss through transpiration	4664.9 <sup>a</sup>	4651.3 <sup>a</sup>	4307.9 <sup>b</sup>	268.5	—
Water intake rate	0.4462 <sup>a</sup>	0.4248 <sup>a</sup>	0.3928 <sup>b</sup>	0.030	0.046
Rate of water loss through faeces	0.0597 <sup>a</sup>	0.0489 <sup>b</sup>	0.0472 <sup>b</sup>	0.009	—
Water absorption rate	0.3879 <sup>a</sup>	0.3759 <sup>a</sup>	0.3456 <sup>b</sup>	0.025	0.038
Transpiration rate	0.2746 <sup>a</sup>	0.2526 <sup>a</sup>	0.2227 <sup>b</sup>	0.028	0.042
Water absorption efficiency	87.0 <sup>a</sup>	88.5 <sup>b</sup>	88.0 <sup>b</sup>	0.650	0.984
Water retention efficiency	29.3 <sup>a</sup>	31.7 <sup>a</sup>	35.6 <sup>b</sup>	3.3	5.0

Mean values: the values of intakes are expressed in Mg/worm/5th instar, the rates are in mg/mg live weight of insect/day and the efficiencies are in percentage. Means in a row (for one parameter) followed by different alphabates (*a*, *b* and *c*) are significantly different  $P < 0.05$  (LSD). NS - Non-significant.

compared with control batch. The shell weight was significantly higher at higher concentrations of Chloramphenicol i.e. at 50 ppm concentration (Table 3).

## DISCUSSION

The food consumption has a direct relevance on the weights of larvae, cocoon, pupae and shell, the independent parameters of consumption and productivity vary depending on the type of nutrition (Shivakumar, 1995) and silkworm breeds (Remadevi *et al.*, 1992). The amount of food consumption/worm/5th instar is more or less same in both control and treated batches. However, in Chloramphenicol treated batches the food assimilated, assimilation rate and assimilation efficiency are higher, which might be due to the influence of antibiotics on the physiology of silkworms. There is a controversy as to whether the Chloramphenicol acts entirely through their anti-bacterial property or by affecting favorably on the physiology and metabolism of the host organism itself (Ayisha Banu, 1991). However, the food utilization by the silkworm larvae is increased due to Chloromycetin administration, which is attributed to increased enzyme activity and influences the utilization of nitrogen, minerals and crude fat upto the extent of 7.28 and 20% respectively which, resulted in vigorous growth of larvae

TABLE 3. Impact of Chloramphenicol on wet food ingested, larval biomass, cocoon weights and their conversion efficiencies in a multi-bivoltine hybrid silkworm race (CB)

Parameter	Control	Chloramphenicol		CD	
		25 ppm	50 ppm	5%	1%
Wet food consumed/ worm/5th instar (gms)	10.6	10.7	10.5	NS	—
Larval biomass (gms)	3.5 <sup>a</sup>	3.8 <sup>b</sup>	3.8 <sup>b</sup>	0.1168	0.1769
Cocoon weight (gms)	1.7 <sup>a</sup>	1.8 <sup>b</sup>	1.9 <sup>b</sup>	0.0341	0.0516
pupal weight (gms)	1.4 <sup>a</sup>	1.5 <sup>b</sup>	1.5 <sup>b</sup>	0.0338	0.0512
Shell weight (gms)	0.2958 <sup>a</sup>	0.3102 <sup>b</sup>	0.3992 <sup>c</sup>	0.0069	0.0104
Cocoon shell ration (%)	17.1 <sup>a</sup>	16.9 <sup>a</sup>	17.7 <sup>b</sup>	0.488	0.7390
Pupation rate (%)	95.3 <sup>a</sup>	98.0 <sup>b</sup>	98.0 <sup>b</sup>	1.3336	2.0195
Larval biomass conversion					
efficiency (%)	33.0 <sup>a</sup>	35.2 <sup>b</sup>	36.3 <sup>b</sup>	1.4273	2.1614
Cocoon conversion	16.4 <sup>a</sup>	17.2 <sup>b</sup>	17.7 <sup>b</sup>	0.6796	1.0291
efficiency (%)					
Pupa conversion	13.5 <sup>a</sup>	14.3 <sup>b</sup>	14.6 <sup>b</sup>	0.5774	0.8743
efficiency (%)					
Shell conversion	2.8 <sup>a</sup>	2.9 <sup>a</sup>	3.1 <sup>b</sup>	0.1501	0.2274
efficiency (%)					

Mean values: Means in a row (for one parameter) followed by different alphabates (a, b and c) are significantly different at  $P < 0.01$  (LSD). NS - Non-significant.

(Shyamala *et al.*, 1960; Verma and Kushwaha, 1971). Govindan *et al.* (1990) have reported that the antibiotics may effect the metabolism by an increase in the feed efficiency or by the activation of enzymes or through hormones, which control and regulate growth. Magadum *et al.* (1996) have reported that the higher food intake does not necessarily result in higher digestibility. Since, the digestibility differed in different breeds, the proportion of food intake to the production of faecal matter also varied (Remadevi *et al.*, 1992) as observed in the present study. The efficiency with which the consumed food was assimilated changed considerably from race to race and from antibiotics to antibiotics at different concentrations (Radha *et al.*, 1981; Ayisha Banu, 1991). Murthy and Sreenivasaya (1953) have reported that the oral administration of Aureomycin, Terramycin and Chloromycetin resulted in increased body weight and nitrogen metabolism as well as silk yield when amino acids were supplemented to such diets. Tayade *et al.* (1988) has observed that all the six antibiotics treated, viz., hostacycline, ledermycin, terramycin, abrimox, cloxacillin and compicillin had a beneficial effect on the silkworm growth and other economic traits over control except fecundity. However, in the present study, Chloramphenicol has influenced the efficiency of food assimilation. Similar observations were made by Ayisha Banu (1991) when, the cross breed (PM × NB<sub>4</sub>D<sub>2</sub>) silkworm larvae were fed with Pencillin and Amphotericin treated

mulberry leaves during 5th instar. The feeding rate, also referred as consumption index explains the rate at which nutrients enter into digestive system. The feeding rate decreased at 50 ppm concentration of Chloramphenicol administration in the present study. On the contrary, it is observed that an increase in feeding rate at 600, 800 and 1000 ppm concentrations of the antibiotics does not help the larvae to enhance the rate of efficiency of conversion of food into body substance but leads to a greater loss due to metabolism (Ayisha Banu, 1991). Similar observations of decreased economic parameters in the bivoltine hybrid silkworm larvae ( $NB_4D_2 \times KA$ ) were recorded when, fed with higher concentration (100 ppm) of Chloramphenicol. However, the larval biomass and cocoon parameters increased in the silkworms fed with varying concentrations of chloramphenicol (40 ppm, 60 ppm and 80 ppm) (Krishnaswami *et al.*, 1980). Takizawa (1990) have reported that the larval development and quantitative characters of the cocoon was apparently inhibited with 10 fold increase of fixed dose (50 ppm) Chloramphenicol. Therefore, the treatment of antibiotic, Chloramphenicol at 50 ppm concentration influences the silkworm larvae to decrease the feeding rate and reduce metabolic loss as much as possible. The inclusion of chloramphenicol in the diet of the silkworm significantly increases food conversion. It was found that the antibiotics fed silkworm larvae had higher amylase activity in the intestine (Govindan *et al.*, 1990; Ayisha Banu, 1991) and increase in the conversion efficiency may be due to better utilization of food by increased enzyme activity (Shyamala *et al.*, 1960, 1976; Verma and Kushwaha, 1971; Radha *et al.*, 1981). However, a comprehensive study of other enzyme system participating in the metabolism may throw more light on the mode of action of the antibiotics.

It is known that the dietary water content in silkworm nutrition has a major influence on its performance (Delvi, 1983). The consumption of dietary water in silkworm has been shown to be directly related to the moisture content of the mulberry leaf and the amount of food intake (Delvi, 1983; Aftab Ahamed *et al.*, 1998). In the present investigation, the water intake and utilization parameters like water absorption efficiency, water retained in the body and retention efficiency were significantly higher in Chloramphenicol treated silkworms whereas, some interrelated parameters like water loss through faeces and transpiration are lower. The silkworms fed with Chloramphenicol treated mulberry leaves have shown, the capacity to absorb more water from the faecal pellets in the rectum leading to higher water absorption efficiency. Similar observations were recorded by Aftab Ahamed (1994) when the cross breed ( $PM \times NB_4D_2$ ) silkworm larvae were forced to feed on fungal infected mulberry leaves.

In the present study, the antibiotic, chloramphenicol may have helped the silkworm larvae in a beneficial way leading to increased conversion and silk synthesis. The increased conversion of food into body matter and cocoon must be due to higher assimilation and increased conversion efficiency of food, though the wet food consumption is more or less similar in control and treated silkworms. This indicates that Chloramphenicol influences the conversion of more food towards shell content as reported earlier (Radha *et al.*, 1981; Tayade *et al.*, 1988; Govindan *et al.*, 1990).

The silk conversion rate is an important factor to be considered while evaluating the economic parameters/traits of a silkworm race. In this context oral administration of Chloramphenicol in the silkworm diet appears to be beneficial by increasing the silk synthesis.

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## Diversity and Habitat Preference of Butterflies in Neyyar Wildlife Sanctuary, South India

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**ABSTRACT:** A study on the diversity and habitat preferences of butterflies was carried out in a south Indian tropical rain forest using transect method. The occurrence of 84 species of butterflies under nine families was revealed. Nymphalidae was the largest among the identified families. Maximum number of endemic species was in undisturbed evergreen forest followed by moderately disturbed moist deciduous forest. Mud puddling and aggregation on excreta were observed in 15 species. Members of the family Danaidae such as the blue tigers (*Tirumala limniace*) and the glassy blue tigers (*Parantica aglea*) were found to concentrate on *Crotalaria scabra* (Fabaceae), a known alkaloid source. © 2001 Association for Advancement of Entomology

**KEYWORDS:** butterflies, habitat of occurrence, habitat specificity, Neyyar Wildlife Sanctuary, tropical rain forest.

### INTRODUCTION

As a signatory of the Convention on Biological Diversity, it is mandatory for India to study in detail and conserve the entire spectrum of its biodiversity. Habitat destruction, ranging from such local effects to more widespread loss of natural habitats over a greater time remains the prime concern of biodiversity conservation. Pressure on natural habitats is likely to increase during the coming decades and hence, several species are likely to face threat on their survival.

Nearly 1500 butterfly species (Gay *et al.*, 1992) are identified from the Indian sub continent, constituting 8.33% of the 18 000 known species of the world (Larsen, 1995). Among insects, butterflies are the most studied (Larsen, 1987a,b,c, 1988; Gunathilagaraj *et al.*, 1997a; Gunathilagaraj, 1997b; Kunte, 1997; Kunte *et al.*, 1999). Most of the Indian butterflies are reported from the Himalayas and from the Western Ghats. Larsen (1987a,b,c, 1988) made a detailed survey of butterflies of the Nilgiri mountains and recorded nearly 300 species. In Kerala, studies on butterflies have

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been carried out in Silent Valley National Park (Mathew and Rahamathulla, 1993) and in Aralam Wildlife Sanctuary (Sreekumar and Balakrishnan, under preparation). The population status of butterflies in any area would help us to understand the status of the ecosystem as they are good indicator species (Kremen, 1992). However, data on the habitat preferences of butterflies and their abundance in diverse habitats of varying ecological parameters are sparse with regard to any region. Hence, the present study deals with the diversity of butterflies in three habitat types of varying disturbance level in the tropical rain forest ecosystem in Neyyar Wildlife Sanctuary in Kerala, South India.

### Study area

Neyyar Wildlife Sanctuary (8°17'–8°53' N, 76°40'–77°17' E), the southern most Protected Area of Kerala State has a total area of 122 km<sup>2</sup> (Fig. 1). Altitude ranges from 90 to 1886 m.s.l. Average annual rainfall is 2800 mm. Maximum mean daily temperature during the hottest month (March) is 35 °C and minimum during the coolest month (January) is 16 °C. Dominant vegetation types of this sanctuary include tropical evergreen forests, tropical moist deciduous forests and moist deciduous forest with undergrowth of grasses. These habitats differed with regard to disturbance levels and percentage of canopy cover. Evergreen forests were comparatively free from disturbance and had more or less closed canopy. Canopy was not closed in moist deciduous forest and was subjected to moderate disturbances. Canopy was open in moist deciduous with undergrowth of grasses and the level of disturbance was at the peak, characterised by cattle grazing, fuelwood collection and forest fire.

### Structural components

The evergreen habitat is characterized by well marked stratification. Common components are *Palaequium ellipticum*, *Mesua ferrea*, *Hopea parviflora*, *Artocarpus hirsuta*, *A. integrifolia*, *Myristica dactyloides*, *Mimusops elengi*, *Vateria indica*, *Calophyllum elatum*, *Cullenia exarillata*, *Acronychia pendunculata*, *Aglaia bourdillonii*, *Antidesma menasu*, *Baccaurea courtallensis*, *Bentinckia codapanna*, *Byrsophyllum tetrandrum*, *Mangifera indica*, *Ficus arnottiana*, *F. hispida*, *Garcinia morella*, *Gordonia obtusa*, *Litsea bourdillonii*, *Mallotus philippinensis*, *Knema attenuata*, *Symplocos macrocarpa*, *Syzigium laetum*, *Trichilia connaroides*, *Canarium strictum*, *Polyalthia fragrans*, *Xanthophyllum flavescens*, *Crotalaria scabra*, *Diotacanthus grandis*, *Macaranga peltata*, *Litsea venulosa*, *Memecylon gracilis*, *M. heyneanum*, *Pandanus thwaitesii*, *Psychotria macrocarpa*, *P. nigra*, *P. nudiflora*, *Tabernaemontana gamblei*, *Gnetum ula*, *Adenia hondala*, *Cayratia tenuifolia*, *Cissus trilobata*, *Toddalia asiatica*, *Millettia rubiginosa*, *Myxopyrum serrulatum*, *Asystasia dalzelliana*, *Chlorophytum laxum*, *Didymocarpus repens*, *Emilia ramulosa*, *Eriocaulon ensiforme*, *Globba ophioglossa*, *Knoxia heyneana*, *Impatiens travancorica*, *I. umbellata*, *Osbeckia virgata*, *Reidia gageana*, *Sonerila rheedii* and *Trichopus zeylanicus*.

Major structural elements of moist deciduous forests are *Artocarpus hirsuta*, *Careya arborea*, *Cleistanthus travancoricus*, *Dillenia pentagyna*, *Bombax ceiba*,



FIGURE 1. Map of Neyyar Wildlife Sanctuary showing transects of the present study.

*Ficus hispida*, *Holigarna arnottiana*, *Terminalia paniculata*, *T. chebula*, *T. bellerica*, *Vitex altissima*, *Pterocarpus marsupium*, *Lagerstroemia* sp., *Emblica officinalis*, *Vitex pinnata*, *Grewia microcos*, *Hedyotis pruinosa*, *Bridelia retusa*, *Helicteres isora*, *Ixora brachiata*, *I. lanceolaria*, *Syzigium cumini*, *Macaranga peltata*, *Melastoma malabathricum*, *Mussaenda laxa*, *Premna glaberrima*, *Psychotria curviflora*, *Butea parviflora*, *Calycopteris floribunda*, *Derris thyrsiflora*, *Holostemma annulare*, *Jasminum rotlerianum*, *Strychnos cinnamomifolia*, *Adenosoma bilabiatum*, *A. subrepens*, *Cyanotis cristata*, *Lindernia* sp., *Torenia travancorica*, *Cyperus castaneus*, *Fimbristylis* sp., *Ochlandra wightii*, *Paspalum scrobiculatum* and *Pennisetum polystachyon*.

Moist deciduous with undergrowth of grasses is formed as a result of retrogression of an originally evergreen type due to frequent annual fires and other human interferences (Chandrasekharan, 1962). Common species identified in this habitat type include *Careya arborea*, *Terminalia paniculata*, *T. chebula*, *Albizia* sp., *Emblica officinalis*, *Pterocarpus marsupium*, *Bridelia retusa*, *Zizyphus oenoplia*, and *Lantana*

*camara*. Ground vegetation is formed of heavy undergrowth of grasses and sedges such as *Cymbopogon citratus*, *Heteropogon* sp., *Themeda* sp., *Eragrostis unioides*, *Pennisetum polystachyon*, *Pycneus puncticulatus*, *Fimbristylis* sp., *Lipocarpha* sp., *Mariscus puncticulatus* and *Saccolipsis* sp.

### Methods

Transect counts were made to monitor butterfly populations during December 1996 to April 1998. Three transects, each with  $1000 \times 10 \text{ m}^2$  were selected at three habitats such as tropical evergreen ( $\geq 500 \text{ m.s.l}$ ), fairly undisturbed moist deciduous forest and disturbed moist deciduous with undergrowth of grasses (between 125–200 m). Each of the transects were visited at least twice per month and all the butterflies observed were recorded transect-wise. The steps were slow but undeviating, covering each transect in about 1 h. Unfamiliar species were collected for identification. Details such as habitat of occurrence and mud puddling were recorded.

### Data analysis

Chi-square analysis was carried out to assess the variation in the abundance of butterflies by taking the cumulative number of each species in each habitat.

### Differentiation diversity (beta diversity)

The beta diversity was estimated using similarity coefficients as a measure of how different or similar a range of habitats or samples are in terms of variety of species found in them. Though several indices exist, Jaccard index was used for the present study as follows:

$$\text{Jaccard index } C_j = j / (a + b - j)$$

Where,

- $j$  = the number of species found in both sites
- $a$  = the number of species in site A
- $b$  = the number of species in site B

## RESULTS

Eighty four species of butterflies classified under nine families were recorded during the present study (Table 1). Most of them were representatives of the Oriental realm while a few were Palaeotropical forms. The family Nymphalidae was the most dominant in terms of number of species.

Among the observed species, 58 (69%) were common in all the habitats, two species (2.4%) were common in moist deciduous forest and in moist deciduous with undergrowth of grasses and 10 (11.9%) species were common in evergreen and in moist deciduous habitats. Eleven species (13%) were observed only in evergreen forests and three species (3.6%) were observed only in moist deciduous forest.

TABLE 1: Butterflies recorded in various habitats in Neyyar Wildlife Sanctuary

Family	Common name	Scientific name	Habitat of occurrence
Acraeidae	Tawny coster <sup>*,2</sup> $\chi^2 = 6.78$	<i>Acraea violae</i> (Fabricius)	EG; MDF
Danaidae	Blue tiger <sup>***</sup> $\chi^2 = 149.6$	<i>Tirumala limniace</i> exoticus Gmelin	EG; MDF; MDF(G)
	Common crow	<i>Euploea core</i> core (Cramer) <sup>1</sup>	EG; MDF; MDF(G)
	Glassy blue tiger	<i>Parantica aglea</i> aglea Cramer	EG; MDF; MDF(G)
	Malabar tree nymph <sup>3</sup>	<i>Idea malabarica</i> (Moore)	EG; MDF
	Plain tiger <sup>*</sup> $\chi^2 = 8.5$	<i>Danaus chrysippus</i> chrysippus (Linnaeus)	EG; MDF; MDF(G)
	Striped tiger	<i>D. genutia</i> genutia (Cramer) <sup>1</sup>	EG; MDF; MDF(G)
Hesperiidae	Brown awl <sup>**</sup> $\chi^2 = 13$	<i>Badamia exclamationis</i> (Fabricius)	EG; MDF; MDF(G)
	Chestnut bob	<i>Iambrix salsala luteipennis</i> Plötz	EG
	Common banded awl	<i>Hasora chromus</i> chromus (Cramer)	EG; MDF; MDF(G)
	Dark palm dart	<i>Telicota ancilla</i> bambusae Moore	EG; MDF
	Giant redaye	<i>Gangara thyrsis</i> thyrsis (Fabricius)	EG; MDF; MDF(G)
	Golden angle	<i>Caprona ransonnetti</i> potiphera Hewitson	EG; MDF; MDF(G)
	Grass demon	<i>Udaspes folus</i> (Cramer)	EG; MDF
	Malabar spotted flat <sup>5</sup>	<i>Celaenorrhinus ambareesa</i> (Moore)	EG; MDF; MDF(G)
	Common redaye	<i>Matapa aria</i> Moore	EG
Lycaenidae	Common cerulean <sup>*</sup> $\chi^2 = 8.3$	<i>Jamides celeno</i> aelianus Fabricius	EG; MDF; MDF(G)

Nymphalidae	Common pierrot*** $\chi^2 = 20.2$	<i>Castalius rosimon</i> rosimon (Fabricius) <sup>1</sup>	EG; MDF; MDF(G)
	Yam fly	<i>Loxura atymnus</i> atymnus Cramer	EG; MDF; MDF(G)
	Angled pierrot*** $\chi^2 = 33.8$	<i>Caleta caleta</i> decidia Hewitson	EG; MDF; MDF(G)
	Red pierrot	<i>Talica niseus</i> niseus Guerin-Meneville	MDF
	Indian cupid	<i>Everes lacturnus</i> syntala Cantlie	EG
	Common Silverline*	<i>Spindasis vulcanus</i> vulcanus (Fabricius)	EG; MDF; MDF(G)
	Pale grass blue	<i>Zizeeria maha</i> ossa (Swinhoe)	EG; MDF; MDF(G)
	Zebra blue	<i>Syntarucus plinius</i> (Fabricius)	EG; MDF; MDF(G)
	Monkey puzzle	<i>Rathinda amor</i> (Fabricius)	EG; MDF
	Angled castor* $\chi^2 = 6$	<i>Ariadne ariadne</i> indica Moore	EG; MDF; MDF(G)
	Common leopard* $\chi^2 = 8.6$	<i>Phalanta phalantha</i> phalantha Drury <sup>1</sup>	EG; MDF; MDF(G)
	Chocolate pansy* $\chi^2 = 8.4$	<i>Precis iphita</i> pluvialis Fruhstorfer <sup>1</sup>	EG; MDF; MDF(G)
	Blue pansy** $\chi^2 = 9.9$	<i>Junonia orithya</i> swinhoei Butler	EG; MDF; MDF(G)
	Yellow pansy* $\chi^2 = 6.5$	<i>J. hierta</i> hierta Fabricius	EG; MDF; MDF(G)
	Lemon pansy	<i>J. lemonias</i> Linnaeus <sup>1</sup>	EG; MDF; MDF(G)
	Peacock pansy	<i>J. almana</i> almana Linnaeus	EG; MDF; MDF(G)
	Grey pansy	<i>J. atlites</i> atlites Linnaeus	EG; MDF; MDF(G)
	Clipper*** $\chi^2 = 32.7$	<i>Parthenos sylvia</i> virens Moore	EG; MDF; MDF(G)
	Southern rustic	<i>Cupha erymanthis</i> maja Fruhstorfer	EG; MDF; MDF(G)

Common nawab	<i>Polyura athamas</i> athamus Drury <sup>1</sup>	EG
Common map	<i>Cyrestis thyodamas</i> indica Evans	EG
Common sailor	<i>Neptis hylas</i> varmona Moore	EG; MDF; MDF(G)
Danaid eggfly** $\chi^2 = 9.7$	<i>Hypolimnas misippus</i> Linnaeus	EG; MDF; MDF(G)
Great eggfly* $\chi^2 = 7.8$	<i>H. bolina</i> jacintha Drury	EG; MDF; MDF(G)
Tamil lacewing	<i>Cethosia nietneri</i> mahratta Felder	MDF; EG
Common Baron* $\chi^2 = 7.9$	<i>Euthalia garuda</i> Moore	EG; MDF; MDF(G)
Grey count	<i>E. lepidea</i> Butler	EG; MDF; MDF(G)
Commander	<i>Moduza procris</i> undifragus Fruhstorfer	EG; MDF; MDF(G)
Common lascar	<i>Pantoporia hordonia</i> hordonia Stoll	EG; MDF; MDF(G)
Tamil yeoman**, <sup>3</sup> $\chi^2 = 10.4$	<i>Cirrochroa thais</i> thais Fabricius	EG; MDF; MDF(G)
Painted lady	<i>Vanessa cardui</i> cardui Linnaeus	MDF
Blue admiral	<i>Kaniska canace</i> viridis Evans	EG; MDF
Common sergeant	<i>Athyma perius</i> perias (Linnaeus)	EG; MDF; MDF(G)
Redspot duke	<i>Euthalia evelina</i> laudabilis Swinhoe	EG
Baronet <sup>2</sup>	<i>Symphaedra nais</i> Forster	EG
Tawny rajah	<i>Charaxes bernardus</i> imna Butler <sup>1</sup>	MDF
Nemeobiidae Plum judy	<i>Abisara echerius</i> prunosa Moore	MDF
Papilionidae Budha peacock	<i>Papilio budha</i> Westwood	MDF; EG
Paris peacock	<i>P. paris</i> tamilana Moore <sup>1</sup>	EG
Common blue bottle** $\chi^2 = 13.7$	<i>Graphium sarpedon</i> teredon Felder & Felder <sup>1</sup>	EG; MDF; MDF(G)
Tailed jay* $\chi^2 = 8.3$	<i>G. agamemnon</i> menides Felder & Felder <sup>1</sup>	EG; MDF; MDF(G)

Pieridae	Southern birdwing* <sup>2</sup> $\chi^2 = 8.7$	<i>Troides minos</i> Cramer	EG; MDF; MDF(G)
	Crimson rose <sup>3</sup>	<i>Pachliopta hector</i> Linnaeus	EG; MDF; MDF(G)
	Common rose	<i>P. aristolochiae</i> aristolochiae Fabricius	EG; MDF; MDF (G)
	Red helen*** $\chi^2 = 21.8$	<i>Papilio helenus</i> daksha Moore	EG; MDF; MDF(G)
	Blue mormon <sup>4</sup>	<i>P. polynnestor</i> Cramer <sup>1</sup>	EG; MDF; MDF(G)
	Lime butterfly	<i>P. demoleus</i> Linnaeus	EG; MDF; MDF(G)
	Fivebar sword tail	<i>Pathysa antipathes</i> Fabricius	EG
	Common mime	<i>Chilasa clytia</i> Linnaeus	EG; MDF; MDF(G)
	Common mormon** $\chi^2 = 12.6$	<i>Papilio polytes</i> Linnaeus <sup>1</sup>	EG; MDF; MDF(G)
	Emigrant	<i>Catopsilia</i> sp. <sup>1</sup>	EG; MDF; MDF(G)
	Common grass yellow* $\chi^2 = 7.8$	<i>Eurema hecabe</i> simulata Moore <sup>1</sup>	EG; MDF; MDF(G)
	Common jezebel* $\chi = 6$	<i>Delias eucharis</i> (Drury)	EG; MDF; MDF(G)
	Common wanderer	<i>Pareronia valeria</i> hippia (Fabricius)	EG; MDF
	Great orange tip* $\chi^2 = 8.8$	<i>Hebomoia glaucippe</i> australis Butler	EG; MDF; MDF(G)
	Psyche** $\chi^2 = 9.5$	<i>Leptosia nina</i> nina (Fabricius)	EG; MDF; MDF(G)
	White orange tip <sup>2</sup>	<i>Ixias marianne</i> (Cramer)	EG
	Yellow orange tip	<i>I. pyrenesesia</i> Fabricius	EG; MDF
Satyridae	Common bush brown	<i>Mycalesis perseus</i> typhlus Fruhstorfer	EG; MDF; MDF(G)
	Dark-brand bushbrown	<i>M. mineus</i> polydecta Cramer	EG
	Glad eye <sup>3</sup> bushbrown*** $\chi^2 = 16.7$	<i>M. patnia</i> junonia Butler	EG; MDF; MDF(G)

Common evening brown*** $\chi^2 = 24.4$	<i>Melanitis leda leda</i> (Drury)	EG; MDF; MDF(G)
Common four ring** $\chi^2 = 10.6$	<i>Ypthima huebneri huebneri</i> (Kirby)	EG; MDF; MDF(G)
Common palmfly* $\chi^2 = 6.2$	<i>Elymnias hypermnestra</i> caudata Butler	EG; MDF; MDF(G)
Common treebrown	<i>Lethe rohria neelgheriensis</i> (Fabricius)	EG; MDF
Nigger	<i>Orsotrioena medus mandata</i> (Fabricius)	EG; MDF; MDF(G)
White bar bushbrown	<i>Mycalesis anaxias anaxias</i> Hewitson	EG

df = 2, \* $P < 0.05$ , \*\* $P < 0.001$ , \*\*\* $P < 0.0001$ , 1. Mud puddlers recorded, 2. Species endemic to India and Sri Lanka, 3. Species endemic to South India and Sri Lanka, 4. Species endemic to Peninsular India and Sri Lanka, 5. Species endemic to India, EG-Evergreen forest, MDF-Moist deciduous forest, MDF(G)-Moist deciduous with undergrowth of grasses.

The abundance of species present was different from habitat to habitat and significant variation was noted for 31 species (Table 1).

Ten (11.90%) species endemic to India and Sri Lanka were recorded from Neyyar Wildlife Sanctuary. All the identified endemic species were observed in evergreen forest, 8 (80%) in moist deciduous forest and 6 (60%) in moist deciduous with undergrowth of grasses. But when the cumulative number of each species per habitat was analysed, the populations of endemics were high in evergreen forest (Fig. 2) and least in moist deciduous with undergrowth of grasses.

Mud puddling and aggregation around excreta of animals were common in some butterfly species. A total of 15 (18.1%) species coming under five families were found to visit damp places or excreta of animals (Table 1). They constituted five Nymphalids, five Papilionids, two Pierids, two Danaids and one Lycaenid. Further, Danaids such as the blue tigers and glassy tigers were found to aggregate in large numbers on *Crotalaria scabra*, a member of the family Fabaceae. These butterflies were found to imbibe the plant juice from the leaves, flowers and flower buds after puncturing the leaves with their claws seen at the tip of forelegs.

Index values revealed that maximum beta diversity was between evergreen and moist deciduous forests (0.81) and maximum dissimilarity was between moist deciduous with undergrowth of grasses and evergreen forests (0.72). Moist deciduous and moist deciduous with undergrowth of grasses show moderate resemblance (0.80).

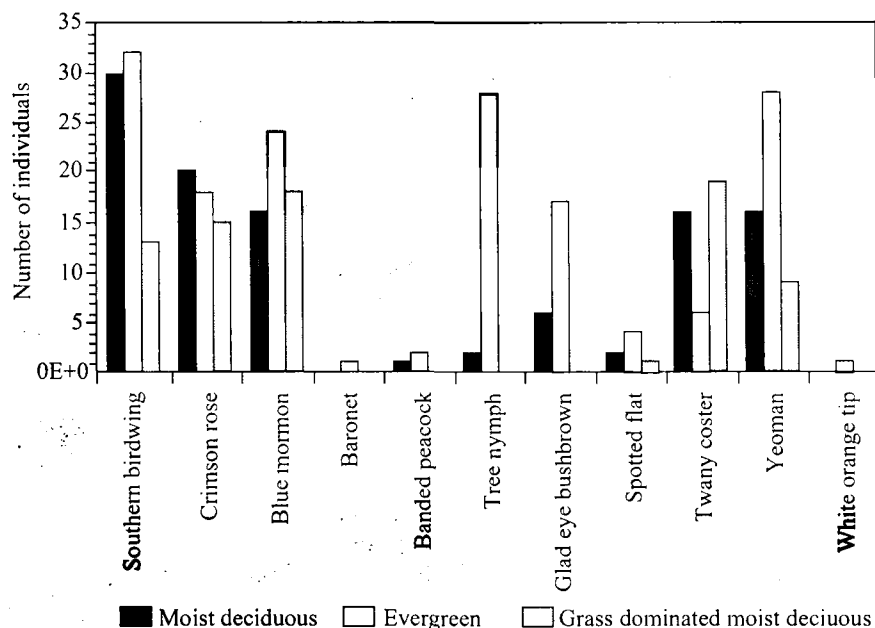


FIGURE 2. Abundance of endemic species of butterflies in different habitats in Neyyar Wildlife Sanctuary.

## DISCUSSION

Zoogeographically, India is part of the Oriental realm and virtually the entire fauna is Oriental in origin (Larsen, 1988). The Palaearctic species are rare represented with few species. The Oriental butterfly fauna of this region is most closely related to that of the Afrotropical region, with which it shares all butterfly families, most subfamilies and about 40 genera (Larsen, 1995). Larsen (1995) suggested that both these regions once constituted a joint evolutionary unit which later split in to two. The family Nymphalidae is the largest family represented with nearly one-third of the known butterflies of the world. This is one of the largest butterfly families in the Western Ghats also. Similar findings have also been reported by Mathew and Rahamathulla (1993) in Silent Valley forest and Sreekumar and Balakrishnan (under preparation) in Aralam Wildlife Sanctuary.

The presence of butterfly species at a particular habitat depends on a wide range of factors, of which the availability of food and climatic conditions are the most important. Other than these, the abundance of larval food plants, conditions suitable for egg laying and suitable flowers for feeding of adults, govern the distribution of butterflies. Further, the abundance of predators and parasitoids and the prevalence of diseases also determine the abundance and density of butterfly populations (Pollard and Yates, 1993). The presence of dark-brand bushbrown and white bar bushbrown in evergreen habitat suggest that they are shade-loving. Satyamurti (1994) stated that Satyrids have preference for shade and they frequent in bushes, grasses and dense

undergrowths or in thick evergreen jungles. However, out of the 84 species recorded, 58 (69%) species were present in all the habitats studied as non-specific in their habitat of occurrence, though the abundance of them varied between habitat types. One obvious suggestion is that disturbances in the form of cattle grazing, forest fire and fuel wood collection as well as variation in the abundance of both larval and adult food plants would have been responsible for this.

During the present study, members belonging to four families were observed to indulge in mud puddling. Adults frequently drink water contaminated with excreta. In addition to mud puddling, species such as blue mormon (*Papilio polymnestor*), blue bottle (*Graphium sarpedon*) and common pierrot (*Castalius rosion*) were also found to have an inclination to elephant dung. Larsen (1987a,b) reported that blue mormon and common pierrot show an affinity to otter droppings in the Nilgiri plateau. This habit help them to satisfy their salt requirements.

Large aggregation of Danaids on plants have been observed during the present study. Members of blue tigers and glassy tigers are found to aggregate on *Crotalaria scabra* by sucking plant juice in May–June and in October–November. Danaid butterflies are well known for their visits to sources of pyrrolizidine alkaloids essential for the males to prime pheromones necessary for successful courtship (Ackery and vane-Wright, 1984 as cited by Larsen, 1987b). Males transfer these compounds to the females which have protective function in eggs (Chapman, 1998). Larsen (1987b) also reported large assemblages of dark blue tiger and *Euploea* spp. on a large yellow *Crotalaria* sp., a known alkaloid source.

The greater community similarity between evergreen and moist deciduous forests may partly result from the more or less similar structure of the vegetation with stratification and canopy cover. Plant diversity and structure affect the viability of many species of terrestrial invertebrate populations (Warren *et al.*, 1997; Launer and Murphy, 1994). Vegetation structure is crucial to butterflies as it has influence on reproduction and thermoregulation (Holl, 1995, 1996). The least beta diversity between evergreen and moist deciduous with undergrowth of grasses reveal that disturbances may also have a profound influence on butterfly populations of the area. Hence butterflies can be considered as valuable ecological indicators (Kremen, 1992). Grazing and forest fire have serious impacts on butterfly populations as cattle overgraze palatable species which would be the larval food plants of some butterflies (Kunte, 1997). The holometabolus life cycle of butterflies exposes them to a wide range of environmental influences including variation in temperature, humidity, light and disturbances (Murphy *et al.*, 1990). Hence, it can be concluded that butterflies are sensitive to disturbances. This is particularly true with respect to endemic species, most of them are habitat specialists.

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## Life Table and Intrinsic Rate of Increase of *Carcelia illota* Curran (Diptera: Tachinidae)

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**ABSTRACT:** The life table and intrinsic rate of increase of *Carcelia illota* Curran (Diptera: Tachinidae) a parasitoid of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) are discussed. The intrinsic rate of increase ( $r_m$ ) was 0.078 and the finite rate of increase ( $\lambda$ ) was 1.081 females per female per day on host. The population was multiplied ( $R_o$ ) by 11.639 times in the cohort generation time (T) of 31.466 days on *H. armigera*. The ovipositing females lived for maximum 10 days.

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**KEYWORDS:** *Carcelia illota*, life table, intrinsic rate of increase, *Helicoverpa armigera*.

### INTRODUCTION

*Carcelia illota* Curran (Diptera: Tachinidae) is a larval ex-pupal parasitoid of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae). Maximum 42 & 52% parasitism have been reported by Chaudhari (1996) and Bilapate (1981), respectively by *C. illota* on *H. armigera*. In recent years, efforts have been made towards natural control of *H. armigera* through its parasitoids (CICR, 1993; King and Coleman, 1989; King and Jackson, 1989; Manjunath *et al.*, 1985). Life table study is one of the useful numerical aids in studying population biology (Southwood, 1978). It is a summary statement on the life of a typical individual of a population helping in studying distribution, determination of age and mortality of organism and from its data the expected life remaining to an individual can be calculated (Price, 1984). The intrinsic rate of increase is the true measure of the reproductive potential of organism.

A perusal of literature on Tachinidae reveals that, the tachinid parasitoids have not been focussed for the life table statistics. This is the first attempt dealing with the study on the population dynamics of *C. illota*. In the present study the life table &

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intrinsic rate of increase have been assessed by analysing the fecundity, based on adult emergence of parasitoid.

#### MATERIALS AND METHODS

*C. illota* adults were reared in the laboratory from the *H. armigera* pupae, which were collected as larvae from the fields of pigeonpea. The rearing of host was carried out on artificial diet developed by Nagarkatti and Satyaprakash (1974) as well as pigeonpea and gram pods. The culture of parasitoid and host were maintained in the BOD incubator at  $25 \pm 1$  °C. However, the adults after emergence were maintained at laboratory temperature ranged from 25 °C to 27 °C for mating and egg laying. The mated females after completion of preoviposition period were released in mating cages of size 1' × 1' × 1'. The cages were specially designed having three glass sides (left, right and top) replaceable with wire mesh (1 mm × 1 mm) frames as per requirement. Of the three sides, left and right sides were of same size (24 cm × 24 cm) and the top was of size (30 cm × 30 cm). The glass and wire frames were adjusted one behind the other on sides or one above the other at the top of the cages so that one of the two can be removed easily. In summer, instead of glass frames wire mesh frames were used so as to provide at least two sides of wire screen that keeps cages well ventilated. However, in winter other small flies may visit through wire screen for food provided to the parasitoid inside the cages; to avoid the nuisance of such intruders glass frames were used. It has been observed during rearing that flies emit some odor, may be pheromone which makes internal environment of the cages unbearable; in that case wire mesh frames were used. This type of adjustment of cages provides sufficient light and ventilation necessary for the diurnal insects like tachinid flies. The front, back and lower sides of the cages were of plywood. The front side was provided with a central hole of 4" diameter with a cloth sleeve for handling the flies. At the bottom wet filter paper was placed to provide required humidity. A cotton swab soaked with 20% honey solution, sugar cubes and sugarcane pieces were placed inside the cage as food for adults. Each female was exposed for 15 host larvae (IV, V and VI instars) one by one on brush, until the death of the parasitoid. There were ten replicates, each replica were containing five females.

The parasitized host larvae were replaced by fresh laboratory reared host larvae. They were held separately in plastic containers of size (5 cm × 3 cm) till the emergence of parasitoid IIIrd instar larvae from host pupae. The parasitoid puparia formed were then kept in plastic containers of size (4.5 cm × 4 cm) until the emergence of adults. The number of females dying on the successive days and number of females produced per female were recorded to find out fecundity. Life table and intrinsic rates of increase of population were determined with the help of fecundity. After the emergence of adult, life table was constructed by using Birch (1948) formula as elaborated by Howe (1953) and Watson (1964), Southwood (1978).

TABLE 1. Age specific fecundity and life table of *C. illota* (Curran)

Pivotal age in days ( $\chi$ )	Age specific longevity ( $l_x$ )	Age specific fecundity ( $m_x$ )	$l_x m_x$	$\chi l_x m_x$
1-31	Immature stages	—	—	—
32	1	1.33	1.33	42.56
33	1	3.33	3.33	109.89
34	1	3.00	3.00	102.00
35	1	1.66	1.66	58.1
36	1	0.66	0.66	23.76
37	0.9	1.00	0.9	33.3
38	0.8	0.66	0.66	20.064
39	0.7	0.33	0.231	9.009
40	0.6	—	—	—
Total	—	11.97	11.639	398.683

## RESULTS AND DISCUSSION

The data on age specific fecundity and life table statistics of *C. illota* is presented in Tables 1 and 2. The ovipositing females lived for a period of 6–10 days with an average of 7.4 days and oviposition period varied from 5–9 days with an average of 6.1 days. The ovipositing females laid maximum number of eggs ( $x = 3.33$ ) on the second day of oviposition. The first adult mortality was noted on the 6th day. The innate capacity of natural increase ( $r_i$ ) was 0.071 while the true intrinsic rate of natural increase ( $r_m$ ) was 0.078 with the finite rate of increase ( $\lambda$ ) being 1.081 females per female per day. The gross reproductive rate ( $m_x$ ) was 11.97 females per female. The population doubling time (DT) was 8.89 days and the weekly multiplication rate of *C. illota* on *H. armigera* was 1.726. Mean length of generation ( $T_i$ ) was 34.25 days that approximated the true generation time (T) of 31.466 days duration which the parasitoid increased at the rate of 11.639 ( $R_o$ ) per generation. This rate of multiplication in *C. illota* is lower in comparison to hymenopteran parasites *Eriborus trochanteratus* (Morely), *Agathis unicolorata* (Shenefelt), parasitoids of *Phthorimaea operculata* (Zeller) (Chandurwar, 1975, 1977); *Xanthopimpla stemmator* (Thunberg) (Basrkar and Nikam, 1981a), *Cotesia flavipes* (Cameron) (Nikam and Sathe, 1983) parasitoids of *Chilo partellus* (Swinhoe). *C. illota* had quicker rate of multiplication in comparison to *Splangia endius* (Walker) (Pteromalidae: Hymenoptera), a parasitoid of *Exorista sorbillans* (Diptera: Tachinidae) (Veeranna and Nirmala, 1992). *H. armigera* had higher rate of multiplication (Bilapate and Pawar, 1980) than its parasitoid.

In the present parasitoid  $r_m$  value observed was low and may have low mortality in nature. This was in line with Smith (1954) that a low  $r_m$  may indicate low mortality in nature and vice versa. In this study  $R_o$  was 11.639 and it was in excess of the one (Southwood, 1978), imply an increasing population with a possibility of bringing about an effective control of its host. It can be also concluded that parasitoids may have lower rate of multiplication than its host. The studies on the life table statistics

TABLE 2. Life table statistics of *C. illota*  
(Curran)

Particulars	Value
$R_0$	11.639
$r_c$	0.071
$r_m$	0.078
$\lambda$	1.081
$T$	31.466 days
$T_c$	34.25 days
DT	8.89 days
Maximum longevity	10 days
Minimum longevity	6 days
Average longevity	7.4 days
Weekly multiplication rate	1.726

of *C. illota* will be helpful in evolving strategies for the management for control of *H. armigera* larvae.

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## **Predatory Potential and Life Parameters of *Cheilomenus* (= *Menochilus*) *sexmaculata* (F.) (Coleoptera: Coccinellidae) in Relation to Energetics of *Aphis gossypii* Glover (Homoptera: Aphididae)**

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**ABSTRACT:** The predatory potential (assessed on the basis of food fed during larval period and by males and females in their adult lives), duration of total post-embryonic development, adult longevity and reproductive capability (reflected by total egg out put) of the aphid predator *Cheilomenus* (= *Menochilus*) *sexmaculata* (F.) were evaluated in relation to the energy content of its prey *Aphis gossypii* Glover. Quantity of food consumed by the predator during its lifetime was relatively less when its prey diet comprised of only adults. A contracted duration of total post-embryonic development and an extension in the adult longevity were observed in this predator when reared on adult aphids. Number of eggs laid by the predator appreciably enhanced, if the aphids preyed upon consisted of adult individuals instead of nymphs. These findings are interpreted as consequences of more energy derived from the ingested adult aphids possessing higher energy content. © 2001 Association for Advancement of Entomology

**KEYWORDS:** predatory potential, post-embryonic development, adult longevity, reproductive efficiency, *C. sexmaculata* and *A. gossypii*.

### **INTRODUCTION**

Several species of ladybird beetles form an important component of any biological control programme involving scale insects and aphids (Agarwala and Choudhuri, 1995; Agarwala *et al.*, 1998) largely due to their high predatory efficiency (Hodek, 1973). The predatory insects show differences in the efficiency in utilizing the available nutrients (Hodek, 1973) and energy from their prey which are eventually reflected in their growth and reproductive performance (Ananthakrishnan *et al.*, 1986; Muthukrisnan and Pandiyan, 1987; Babu, 1991, 1993, 1999). In addition, different morphs of several species of aphids also tend to alter the growth, egg laying sequence and total egg out put among coccinellids (Wiperfurth *et al.*, 1987). In the light of such available information, an effort has been made in this work to examine the predatory potential (in terms of prey consumption), post embryonic developmental

duration, adult longevity, and reproductive performance (determined on the basis of total egg out put) of one of the predatory coccinellid, *Cheilomenes sexmaculata* F when offered nymphs or adults of the aphid *Aphis gossypii* Glover as food wherein the energy content of the ingested prey varied.

## MATERIALS AND METHODS

Larvae and adults of *C. sexmaculata* were collected from leaves of cotton infested by *A. gossypii* in fields, in and around the City of Chennai, and they served as starting pointy for establishing a standard laboratory stock culture. The beetles were reared on nymphs and adults of the aphid (renewed daily) in glass troughs (25 × 10 cm), at 25 °C, 14 L : 10 D, and 65 ± 5% RH as mentioned above. The troughs were covered on the top with muslin cloth.

Duration of total post-embryonic development and adult longevity of this predatory coccinellid were investigated by rearing them separately on nymphs and adults of *A. gossypii*. For this, eggs of *C. sexmaculata* deposited on aphid infested host leaves were taken as such from the laboratory culture and kept in separate plastic containers (7.5 cm × 2.5 cm). The petioles of these leaves were plugged with wet cotton to avoid desiccation till hatching of eggs. Thereafter two separate batches (each comprising 20 individuals) of neonate larvae were reared on aphid nymphs/adults, replenished everyday in plastic containers (dimensions same as given above). Total post-embryonic developmental period (covering the entire larval and pupal durations) of the predator raised on nymphs or adults of aphids were recorded.

### Predatory potential

For evaluating the coccinellid's predatory potential, the daily consumption of nymphs or adults of aphids (prey) by different larval stages of the predator was assessed till pupation. Prey density (nymphs/adults) was always maintained uniform and consisted of 75-individuals/predator until pupation. The number of prey consumed by the predator during its total larval period was also noted.

### Reproductive performance

Total egg output and percent reproductive efficiency were also recorded for females coupled with males (both >24 h old) in single pairs for one complete generation. Reproductive efficiency (RE, expressed in percent) was calculated following the method adopted by Muthukrisnan and Pandiyan (1987)

$$\text{RE} = \frac{\text{Energy allocated to egg production (J/insect)}}{\text{Total energy assimilated during the adult phase}} \times 100$$

J = unit of energy (Jules).

All experiments were conducted under the same conditions as described for the maintenance of laboratory culture and each one was run five times with separate samples of predator and prey individuals.

TABLE 1. Predatory Potential (based on number of prey individuals ingested daily) of *Cheilomenes sexmaculata* on *Aphis gossypii*

Prey stage	Predator stage					
	Larval Stage				Adult	
	I instar	II instar	III instar	IV instar	Male	Female
Nymphs	18.2 ± 0.33 <sup>a</sup>	26.0 ± 0.84 <sup>a</sup>	32.6 ± 0.60 <sup>a</sup>	37.6 ± 0.45 <sup>a</sup>	36.5 ± 0.53 <sup>a</sup>	56.6 ± 0.79 <sup>a</sup>
Adults	14.6 ± 0.35 <sup>b</sup>	18.4 ± 0.59 <sup>b</sup>	26.0 ± 0.74 <sup>b</sup>	36.0 ± 0.74 <sup>b</sup>	33.2 ± 0.66 <sup>b</sup>	44.0 ± 1.26 <sup>b</sup>

Values represent ±SE of 5 replicates. Values followed by similar superscripts within column are not statistically significant ( $P > 0.05$ ) by ANOVA.

TABLE 2. Larval–pupal duration and adult life span of *Cheilomenes sexmaculata* fed on nymphal or adult individuals of *Aphis gossypii*

Prey stage	Larval–pupal duration (days)	Longevity (days)
Adults	17.0 ± 0.28 <sup>a</sup>	32.6 ± 0.35 <sup>a</sup>
Nymphs	15.4 ± 0.45 <sup>b</sup>	36.4 ± 0.45 <sup>b</sup>

Values represent mean ± SE of five replicates. Means in column having different alphabet are significantly different ( $P > 0.05$ ) by ANOVA.

### Prey consumption and energetics

Consumption and utilization of prey, in terms of latter's energy content, were estimated following the gravimetric method described by Waldbauer (1968). Accordingly, food consumption (C) egestion (FU) and growth (P) were calculated and the energy content of the different stages have been estimated using a semi micro-bomb calorimeter (Parr. Inc. Co., USA). Bioenergetics parameters were estimated following IBP formula,  $[C = FU + P + R]$  of Petruszewicz and Macfadyen (1970). For bioenergetic estimations, 10 mg of each of the two stages of the aphid (nymphs/adults) was taken and such determinations were done 5 times.

Assimilation was calculated as the difference between food consumed and the total faeces voided ( $A = C - FU$ ). Metabolism was calculated as the difference between assimilated energy and growth.

## RESULTS

### Predatory potential

In *C. sexmaculata*, the predatory potential (based on number of prey individuals consumed daily) was far greater in adults (more so in females) than larvae (Table 1). Even in the larval phase, the predatory efficiency of IV instar grubs exceeded that of the adult males. But nymphs were always ingested in greater number than adults by the predator. Thus the predatory potential of the coccinellid appears to be closely

linked with the stage of the prey. A grub of *C. sexmaculata* during its development fed a total of 295.96 nymphs or 243.62 adults (mean value) of *A. gossypii*. The predatory potential, in terms of the total number of aphids devoured as nymphs, during each larval instars were 38.9, 48.4, 68.1, 92.6 respectively (mean values). It was relatively less when the predator's food consisted of adult aphids.

#### Post-embryonic developmental duration and adult life span

In *C. sexmaculata*, grubs reared on adult aphids completed their post-embryonic development relatively faster (Table 2) and this was found to be influenced by the energy content of the stage of aphids (51.14 J for nymphs and 85.77 J for adults not given in Table 2) preyed upon. The resultant predators lived longer if their maintenance was continued on adult aphids.

#### Reproductive efficiency (%)

Adult aphids ingested by the predator provided 178.4 J of energy to females resulting in maximum egg deposition (Table 3), which in turn indicated a high reproductive efficiency (46.4%)

Longevity and reproductive efficiency of *C. sexmaculata* showed a positive correlation with energy content of the two different stages of aphids, which, however, was negative when correlated with duration of post-embryonic development of the predator (Fig. 1).

### DISCUSSION

Decreased intake of adult individuals of *A. gossypii* by *C. sexmaculata* during larval period or in adult stage, like in another carnivorous coccinellid *Nephus regularis* Sicard feeding on the striped mealy bug (Rawat and Modi, 1969), is presumably due to fulfilment of dietary requirement of the predator following consumption of less number of such large sized prey individuals possibly with superior nutritional value.

Shortened post-embryonic development period and extended longevity of *C. sexmaculata* reared on adults of *Aphis gossypii* agree with the earlier findings of Fisher (1963), Bhat (1981), Mani and Thantordarya (1987) on the mealy bug predator *Cryptolaemus montrouzeri* Muls. Such observations in the case of *C. sexmaculata* appear to be a consequence of higher energy content of these prey individuals (Babu, 1991, 1993, 1999) provided to the predator. A steady increase in the quantum of energy obtained by this coccinellid species with progress in its post-embryonic development can be attributed to higher energy demand necessary for growth during metamorphosis in this insect.

Enhanced total egg out put by females of *C. sexmaculata* ingesting adults of *A. gossypii* was a phenomenon similar to that when this predator fed on *Schizaphus graminum* (Rondani) (Campbell *et al.*, 1980) adult aphids. This can also, in respect of the findings obtained in this study, perhaps be a sequel to procurement of more energy from *A. gossypii* adult prey individuals by the predator during oviposition.

TABLE 3. Bioenergetics of *Cheilomenes sexmaculata* fed on different stages of *Aphis gossypii*

Prey stage	Predator stage				
	Larval Stage			Adult	
	I instar	II instar	III instar	IV instar	
Nymphs	3.28 ± 0.08 <sup>a</sup>	4.20 ± 0.14 <sup>a</sup>	13.48 ± 0.23 <sup>a</sup>	19.20 ± 0.14 <sup>a</sup>	Male 127.68 ± 1.86 <sup>a</sup> Female 149.2 ± 0.86 <sup>a</sup> # 224 ± 3.22
Adults	5.6 ± 0.12 <sup>b</sup>	7.80 ± 0.11 <sup>b</sup>	18.72 ± 0.10 <sup>b</sup>	26.52 ± 0.19 <sup>b</sup>	Male 166.6 ± 117 <sup>b</sup> Female *178.8 ± 1.0 <sup>b</sup> #274 ± 2.24

Values represent ±SE of 5 replicates. \* Includes 84.7 J as egg energy. # Total egg out put/female.

Values followed by similar superscripts within column are not statistically significant ( $P > 0.05$ ) by ANOVA.

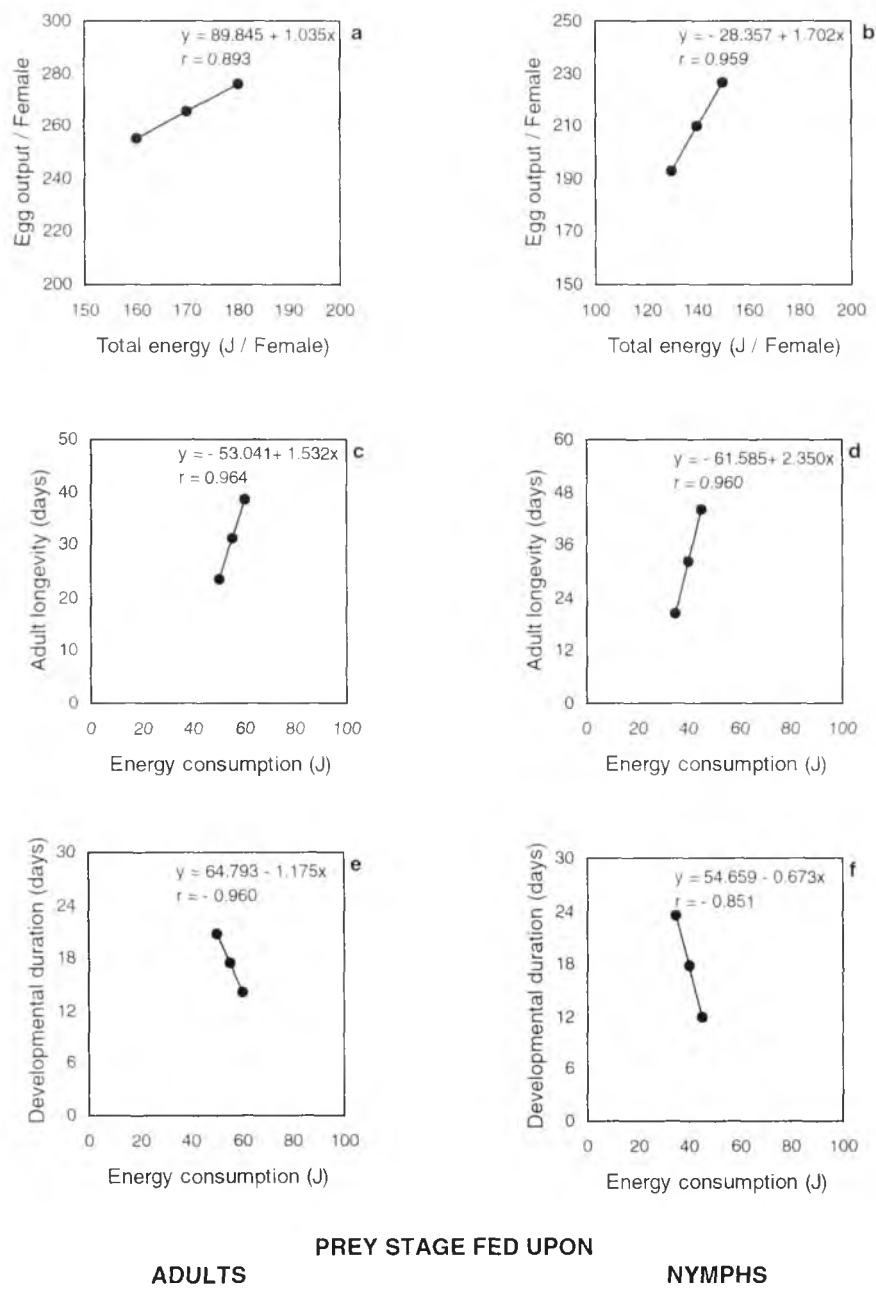


FIGURE 1.

From these observations concerning coccinellid-aphid interaction, it can be concluded that nutritional quality (in terms of energy content) of *A. gossypii* preyed by *C. sexmaculata* plays a major role in influencing the predatory potential, post-embryonic development, adult life-span and egg out put of the latter.

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## Compatibility of Entomopathogenic Nematodes (Steinernematidae, Heterorhabditidae: Rhabditida) with Selected Pesticides and Their Influence on Some Biological Traits

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**ABSTRACT:** The effect of five pesticides including one botanical pesticide on the biological traits viz., activity, penetration rate, infectivity and progeny production of two *Steinernema bicornutum* and two *Heterorhabditis indica* isolates was assessed by using *Galleria mellonella* L. larvae. The results indicated that the infective juveniles of both the genera tolerated most of the chemicals tested but the response to different pesticides appears to be variable. The inactivity per cent increased with increased time and concentration of pesticide and it was less than 40% and 35% for heterorhabditids and steinernematids respectively at field recommended dosages. In general, infectivity of pesticide exposed infective juveniles was not adversely affected. Exposure for 72 h to pesticides impaired the penetration rate of *S. bicornutum* isolates (30–40%) while additive response was observed in *H. indica* isolates. Overall, no additive or synergistic response was observed in progeny production of pesticide exposed infective juveniles. Among the pesticides, mancozeb and neem were safe to all the nematode populations while the latter was deleterious to *H. indica* PDBC 13.3. Fifteen out of twenty combinations tested were compatible and may be included in any IPM schedule. The improvement of susceptible population is highlighted. The entomopathogenic nematodes tested above may be viable candidates for an integrated approach. © 2001 Association for Advancement of Entomology

**KEYWORDS:** *Steinernema bicornutum*, *Heterorhabditis indica*, pesticides, compatibility, biological traits.

### INTRODUCTION

With the increasing concern over pesticide resistance in insects and residues in environment, Biointensive Integrated Pest Management (BIPM) has emerged out as an important strategy for control of insect pests. In the present scenario none of

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the strategies can be used as a sole component for successful management. Use of chemical pesticides and biocontrol agents in IPM has received much interest in recent years (Hara and Kaya, 1982). The entomopathogenic nematodes (EPN), a highly competitive biocontrol agent have been used successfully to suppress insect pests in several crops. For any biocontrol agent to fit into IPM strategy, it should be compatible with other methods of control (Kaya, 1985).

Although the compatibility of some *Steinernema* spp. (Rao *et al.*, 1975; Hara and Kaya, 1983) and *Heterorhabditis* spp. (Rovesti and Deseo, 1990) has been examined, only activity and infectivity of exposed infective juveniles were considered. This study has been extended to consider other biological traits such as penetration rate and progeny production. Moreover, comparison of the biological traits of pesticide exposed infective juveniles of *Steinernema bicornutum* and *Heterorhabditis indica* isolates has been done. The effect of botanical pesticide (Neem oil) on EPN is also studied.

## MATERIALS AND METHODS

### Nematode and insect culture

*S. bicornutum* PDBC 2.1 and PDBC 3.2 and *H. indica* PDBC 6.71 and PDBC 13.3 were isolated from soil samples drawn from different crop ecosystems. The populations were reared on final instar larvae of *Galleria mellonella*. The infective juveniles of *S. bicornutum* and *H. indica* isolates were stored at 8 °C and 24 °C respectively before use. The test insect, *G. mellonella* were reared on artificial diet as per procedure described by Singh (1994).

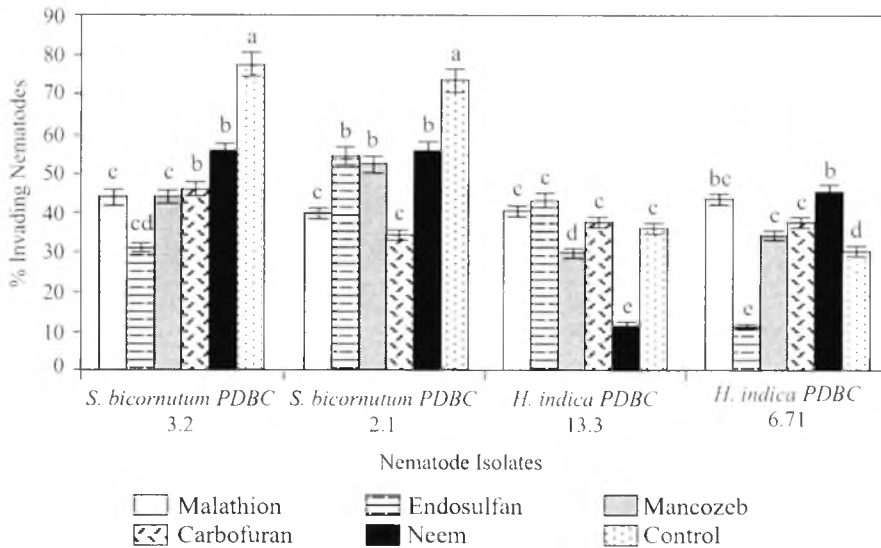
### Effect of pesticides on the biological traits of EPN

#### *Assay in aqueous suspension*

The commonly used commercial formulations of insecticides (Endosulfan, Malathion), fungicide (Mancozeb), nematicide (Carbofuran) and botanical pesticide (Neem oil) were selected for evaluation. The aqueous suspension of the pesticides was prepared based on the recommended field rates (Endosulfan - 1.6 ppm, Malathion - 1.2 ppm, Mancozeb - 2600 ppm, Carbofuran - 3000 ppm and Neem - 1.5 ppm) with one higher and one lower concentration of the recommended field rate as treatments and a control (exposed to distilled water). The activity assay was done for the above mentioned populations as described by Forschler *et al.* (1990).

#### *Other assays*

Petriplate bioassay method was followed to determine the effect of exposed IJs on other biological traits of nematode populations. After the prescribed exposure period (72 h), nematode suspensions of *S. bicornutum* and *H. indica* isolates from each petriplate were centrifuged at 600 rpm for 3 minutes separately. The resultant supernatant was decanted and replaced by distilled water. This procedure was repeated



The means with the same letter do not differ significantly by DMRT ( $P=0.05$ )

FIGURE 1. Penetration rate of pesticide exposed infective juveniles in *Galleria mellonella*.

thrice to wash off the pesticide residues from nematodes. Infectivity of exposed and washed infective juveniles was evaluated at a standard dosage level of 100 infective juveniles/larva of *G. mellonella* and replicated five times. Mortality was recorded 72 h post inoculation.

The same set of procedure was followed to study the effect on penetration rate and progeny production. The infective juveniles exposed to recommended field dosages of pesticides alone were studied. The infected larvae of *G. mellonella* were cut open 72 h post inoculation and the percentage of invaded nematodes was calculated for penetration rate. For progeny production, white trap method was followed (Woodring and Kaya, 1988).

The data were analysed statistically using DMRT, after transforming the values.

## RESULTS AND DISCUSSION

### *Effect on activity*

*S. bicornutum* PDBC 2.1 was found to be more compatible (<20% inactivity) with all the pesticides tested compared to *S. bicornutum* PDBC 3.2 (Table 1). Among the chemical pesticides, Carbofuran (3000 ppm) impaired the activity of steinerne-matids (13.35%). Zimmerman and Cranshaw (1990) recorded more sensitivity of *Neoplectana bibionis* to carbamate even at 400 ppm. Mancozeb and neem were found to be relatively safe even at 120 h and twice the recommended dosages while

TABLE 1. Percentage inactivity of *Steinernema bicornutum* isolates upon exposure to selected pesticides in aqueous solutions

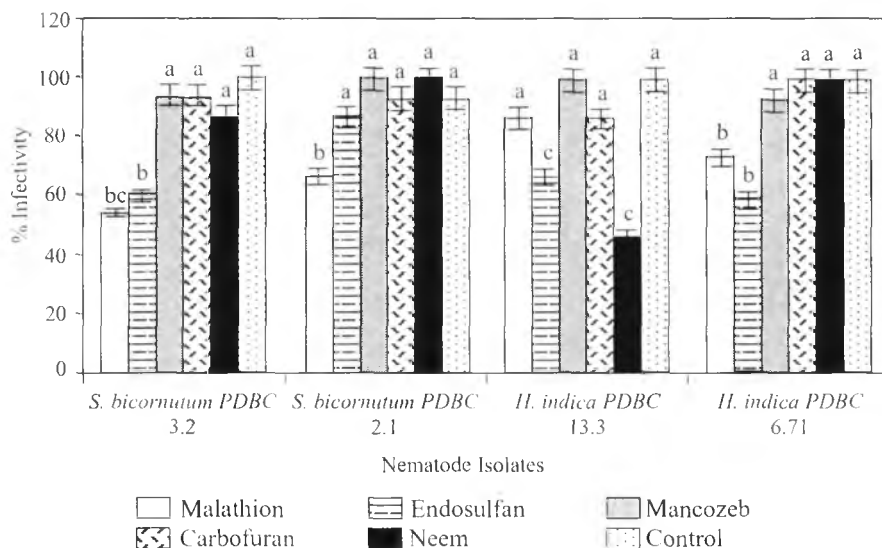
Pesticide	Exposure Time (h)*				Pesticides concentration ( $\mu\text{g}/\mu\text{l}$ )**					
	<i>S. bicornutum</i> PDBC 2.1		<i>S. bicornutum</i> PDBC 3.2		<i>S. bicornutum</i> PDBC 2.1		<i>S. bicornutum</i> PDBC 3.2		<i>S. bicornutum</i> PDBC 3.2	
	24	72	24	72	1/2N	N	1/2N	N	1/2N	2N
Endosulfan	4.44 (12.82) <sup>f</sup>	7.26 (16.17) <sup>h</sup>	12.89 (21.41) <sup>g</sup>	34.52 (36.27) <sup>d</sup>	4.29 (12.60) <sup>i</sup>	7.41 (16.28) <sup>g</sup>	12.89 (21.42) <sup>f</sup>	17.48 (25.09) <sup>e</sup>	26.96 (31.58) <sup>d</sup>	46.06 (43.02) <sup>a</sup>
Malathion	7.41 (16.31) <sup>h</sup>	10.81 (19.63) <sup>g</sup>	17.33 (24.97) <sup>f</sup>	33.02 (35.37) <sup>d</sup>	6.37 (15.17) <sup>h</sup>	10.81 (19.57) <sup>f</sup>	18.37 (25.72) <sup>e</sup>	14.81 (23.02) <sup>f</sup>	30.89 (34.05) <sup>c</sup>	38.89 (38.86) <sup>b</sup>
Mancozeb	3.24 (11.02) <sup>f</sup>	6.52 (15.31) <sup>f</sup>	11.85 (20.56) <sup>g</sup>	9.63 (18.53) <sup>g</sup>	4.44 (12.83) <sup>f</sup>	7.11 (15.99) <sup>h</sup>	10.07 (18.97) <sup>g</sup>	4.74 (13.21) <sup>f</sup>	8.88 (17.82) <sup>g</sup>	15.70 (23.66) <sup>e</sup>
Carbofuran	7.56 (16.42) <sup>h</sup>	14.37 (22.63) <sup>f</sup>	21.48 (27.94) <sup>e</sup>	39.92 (39.43) <sup>c</sup>	8.59 (17.51) <sup>g</sup>	13.93 (22.31) <sup>f</sup>	20.89 (27.48) <sup>d</sup>	25.04 (30.34) <sup>d</sup>	34.81 (36.32) <sup>b</sup>	49.34 (44.91) <sup>a</sup>
Neem	0.00 (4.05) <sup>k</sup>	0.29 (4.86) <sup>k</sup>	6.81 (15.54) <sup>hi</sup>	0.88 (4.05) <sup>f</sup>	1.48 (7.53) <sup>f</sup>	2.66 (9.71) <sup>f</sup>	2.96 (10.62) <sup>f</sup>	1.18 (7.00) <sup>k</sup>	1.88 (8.33) <sup>f</sup>	2.22 (9.14) <sup>f</sup>
Control	1.33 (7.32) <sup>k</sup>	3.11 (9.86) <sup>f</sup>	4.00 (10.87) <sup>f</sup>	3.55 (11.37) <sup>f</sup>	2.96 (10.55) <sup>f</sup>				2.81 (10.25) <sup>f</sup>	

\*CD( $P = 0.05$ ). Pesticide  $\times$  Nematode  $\times$  Time = 2.89. \*\*CD( $P = 0.05$ ) Pesticide  $\times$  Nematode  $\times$  Concentration = 2.47. Figures in parentheses are arc sin transformed values. N - Normal field recommended dosage. Means followed by the same letter do not differ significantly.

TABLE 2. Percentage inactivity of *Heterorhabditis indica* isolates upon exposure to selected pesticides in aqueous solutions

Pesticide	Exposure Time (h)*			Pesticides concentration ( $\mu\text{g}/\mu\text{l}$ )**					
	<i>H. Indica</i> PDBC 6.71			<i>H. Indica</i> PDBC 13.3			<i>H. Indica</i> PDBC 6.71		
	24	72	120	24	72	120	1/2N	N	2N
Endosulfan	21.63 (28.02) <sup>d,e</sup>	36.28 (37.32) <sup>e</sup>	52.67 (46.82) <sup>d</sup>	10.52 (19.33) <sup>f</sup>	18.04 (25.42) <sup>e</sup>	26.30 (31.09) <sup>d</sup>	24.67 (30.07) <sup>d</sup>	37.18 (37.86) <sup>b</sup>	48.14 (44.22) <sup>d</sup>
Malathion	25.04 (30.30) <sup>d</sup>	43.01 (41.26) <sup>b</sup>	51.11 (45.93) <sup>d</sup>	6.37 (15.18) <sup>g</sup>	16.50 (24.30) <sup>e</sup>	23.70 (29.47) <sup>d</sup>	32.96 (35.32) <sup>e</sup>	40.29 (39.62) <sup>b</sup>	46.22 (43.09) <sup>d</sup>
Mancozeb	0.59 (5.67) <sup>i</sup>	5.03 (13.58) <sup>g</sup>	14.50 (22.77) <sup>e,f</sup>	4.59 (13.02) <sup>g</sup>	11.01 (19.72) <sup>f</sup>	15.85 (23.80) <sup>e</sup>	4.14 (12.20) <sup>h</sup>	6.22 (14.97) <sup>h</sup>	9.78 (18.65) <sup>g</sup>
Carbofuran	1.18 (6.62) <sup>i</sup>	2.96 (10.26) <sup>h</sup>	10.66 (19.42) <sup>f</sup>	10.07 (21.69) <sup>f</sup>	24.87 (30.23) <sup>d</sup>	31.26 (34.21) <sup>e</sup>	2.37 (9.31) <sup>i</sup>	3.55 (11.44) <sup>i</sup>	8.30 (17.07) <sup>g</sup>
Neem	0.0 (4.05) <sup>i</sup>	0.30 (4.86) <sup>i</sup>	1.48 (7.53) <sup>h</sup>	1.48 (7.43) <sup>h,i</sup>	33.18 (35.47) <sup>e</sup>	40.89 (40.03) <sup>b</sup>	0.59 (5.67) <sup>i</sup>	0.29 (4.86) <sup>j</sup>	0.89 (6.19) <sup>j</sup>
Control	0.0 (4.05) <sup>i</sup>	0.89 (6.23) <sup>i</sup>	1.33 (6.90) <sup>i</sup>	0.00 (4.05) <sup>i</sup>	2.66 (9.74) <sup>h</sup>	6.22 (14.91) <sup>g</sup>	0.74 (6.14) <sup>j</sup>	2.96 (10.62) <sup>j</sup>	

\*CD( $P = 0.05$ ) Pesticide  $\times$  Nematode  $\times$  Time = 3.01. \*\*CD( $P = 0.05$ ) Pesticide  $\times$  Nematode  $\times$  Concentration = 2.89. Figures in parentheses are arc sin transformed values. N = Normal field recommended dosage. Means followed by the same letter do not differ significantly.



The means with the same letter do not differ significantly by DMRT ( $P=0.05$ )

FIGURE 2. Infectivity of pesticide exposed infective juveniles against *Galleria mellonella*.

the latter was deleterious to *H. indica* PDBC 13.3 alone with 26.59% inactivity as recorded by Welch (1971) on *N. carpocapsae*.

*H. indica* PDBC 13.3 was more tolerant compared to PDBC EN 6.71. Endosulfan (1.6 ppm) and Malathion (1.2 ppm) had discernible effect on *H. indica* PDBC 6.71 (35–40%) while Carbofuran was toxic to *H. indica* PDBC 13.3. This is in agreement with findings of Rovesti and Deseo (1990) that organophosphorus (OP) compounds and Endosulfan caused a reduction in the movement of infective juveniles. Generally, pesticides in a solution varied in effect on entomophilic nematode strains as observed by Zimmerman and Cranshaw (1990).

#### Effect on penetration rate

The penetration rate of steinernematids (Fig. 1) had impaired when exposed to pesticides for 72 h. The effect was more felt in the combination of *S. bicornutum* PDBC 3.2 with Endosulfan and Malathion. On the other hand, infective juveniles of *H. indica* PDBC 13.22 exposed to neem had lowest penetration rate (12%). Hara and Kaya (1983) reported the toxicity of some selected OP and carbamate pesticides but not the infective juveniles of *N. carpocapsae*.

#### Effect on infectivity

The infectivity of pesticides treated infective juveniles was not affected adversely (Fig. 2) except a few combinations as mentioned above were incompatible with

TABLE 3. Progeny production of pesticides exposed *Steinernema bicornutum* and *Heterorhabditis indica* isolates

Nematode	% difference in progeny production over control of pesticide exposed nematodes				
	Endosulfan	Malathion	Carbofuran	Mancozeb	Neem
<i>S. bicornutum</i> PDBC 3.2	-66.75	-38.46	-5.38	-16.92	-14.33
<i>S. bicornutum</i> PDBC 2.1	-35.59	-21.66	-19.01	-1.56	-54.64
<i>H. indica</i> PDBC 6.71	-35.30	-66.67	-20.59	-27.61	-15.25
<i>H. indica</i> PDBC13.3	-46.74	-31.14	-25.17	-12.72	-15.94

reduction of 40–50% infectivity. Rovesti and Deseo (1990) observed that the effect of some OP compounds and Endosulfan on the infectivity of *S. carpocapsae* and *S. feltiae* was negligible.

#### *Effect on progeny production*

The progeny production of pesticides exposed nematodes in *G. mellonella* larvae was impaired (Table 3). The OP compounds were toxic to all the nematode populations with reduction of 30–72%. The progeny production was drastically reduced (54–64%) in neem exposed IJs of *H. indica* PDBC 13.3 Hara and Kaya (1982) observed that all OP and carbamate tested affected the development and reproduction of *N. carpocapsae*. *N. carpocapsae* did not develop in *G. mellonella* larvae when the IJs were treated with OP and nematicides.

Out of twenty combinations tested, five were incompatible *viz.*, Endosulfan + *S. bicornutum* PDBC 2.1, PDBC 3.2 and *H. indica* PDBC 6.71, Malathion + *S. bicornutum* PDBC 2.1 and neem + *H. indica* PDBC 13.3 based on the effect on all the biological traits. Rao *et al.* (1975) tested the toxicity of various OP insecticides to infective juveniles in water suspension. The incompatible strains can be improved by exposing infective juveniles to lower concentration of pesticides and increasing the concentration for exposure of subsequent generations of tolerated strains till the desired result is achieved.

In our study a few pesticides had adverse effect on activity of infective juveniles (Carbofuran and *S. bicornutum* isolates) however the other biological traits were not affected and are hence compatible. This is in agreement with the findings of Rovesti and Deseo (1990). This study projects the compatible combinations that may be suitable for co-application. Chemical insecticides and EPN offer different but potentially compatible approaches to suppress insect populations (Nishimatsu and Jackson, 1998). The entomopathogenic nematodes may seek out the host in inaccessible areas where pesticides may not act. The entomopathogenic nematodes

tested above may be viable candidates for this integrated approach. Studies on the residual effects of the insecticides in the field, which directly affects the recycling of entomopathogenic nematodes, are to be explored.

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## Influence of Semisynthetic Diets on the Total Protein and Lipid Content of the Larvae of *Spodoptera litura* (Fab.)

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**ABSTRACT:** The larvae of *Spodoptera litura* were analysed for their total protein and total lipid content to find out the effect of diets containing different steroids, vitamins, aminoacids, plant extracts and oils at various concentrations. The highest protein content was recorded in diets with stonazolol 0.004 per cent, vitamin C 0.5 per cent, tryptophan 0.1 per cent and *Tribulus terrestris* 10 per cent among the steroids, vitamins, aminoacids and plant extracts respectively. Protein content was more where the larval weight increased. Based on observations on total lipid content of the larvae reared on different diets showed that the treatments with steroids and oils were on par with control indicating that they influence the sterol metabolism as that of the cholesterol in the control. The larvae reared on diets with vitamins, aminoacids and plant extracts recorded lesser lipid content compared to control as their incorporation in the diet did not influence the sterol metabolism.

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**KEYWORDS:** *Spodoptera litura*, semi-synthetic diets, protein, lipid, larval growth.

### INTRODUCTION

Biochemical analysis of larvae are carried out for several purposes. Changes in carbohydrate contents in the body of the host insects like *Heliothis virescens* during CPV infections (Morris, 1968; Thompson and Sikorowski, 1986) have already been reported. During NPV infection, the glycogen and glucose contents of target tissues decreased, perhaps concurrently increasing the haemolymph of health and diseased larvae of Japanese beetle, *Popilliae japonica* have been characterised and compared (Bennett and Shotwell, 1970). Rajanna *et al.* (1994) reported that the total protein concentrations was 1.2 fold higher in bivoltine strain of the silkworm, *Bombyx mori* than multivoltine strain.

JHA (hydroprene) treated larvae showed accumulation of haemolymph protein and

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increased the total aminoacid concentration in haemolymph (Zeenath and Nair, 1994). As the maturation of pathogen proceeds, the carbohydrate content decreased, because the carbohydrates like glycogen and glucose were utilized possibly for growth, multiplication and maturation of pathogen (Ingalthalli *et al.*, 1995). In the present study, the effect of different steroids, vitamins, aminoacids and plant extracts were tested to find out their influence on the larval protein and lipid content, as indicative of growth factors.

## MATERIALS AND METHODS

### Semisynthetic diet

The semi-synthetic diet was prepared using bengalgram flour as the major ingredients and the following were the constituents of the diet.

Water	:	720.0 ml
Bengalgram flower	:	150.0 g
Yeast tablets	:	30.0 g
Wesson's salt mixture	:	7.2 g
Sorbic acid	:	2.0 g
Formaldehyde 40%	:	1.0 g
Agar-agar	:	12.8 g
10% choline chloride	:	0.2 ml
Enriched vitamin drops	:	2.0 ml
Ascorbic acid	:	3.2 g
Streptomycin sulphate	:	40.0 mg

To 360 ml of boiling water, the bengalgram flour was added and mixed well. To this, the Wesson's salt mixture, methyl para hydroxy benzoate, yeast tablets and sorbic acid were added and the mixture was homogenized. With the remaining amount of water, the agar was boiled. After cooling to about 20–30 °C, it was added to the mixed diet and homogenized. Finally, streptomycin sulphate, ascorbic acid, enriched vitamin drops and 10 per cent choline chloride were added and homogenized by thorough mixing. The diet was then poured to the individual plastic containers for rearing the larvae.

### Laboratory experiments

Lab evaluations were carried out to study about the quantitative requirement of the steroids preferred by the larvae, different quantities of individual steroids were added to the basic diet keeping the other ingredients as constant. The concentrations of steroids used in the diets are 0.001, 0.002, 0.004, 0.006, 0.008 and 0.01% to maintain 1, 2, 4, 6 and 10 mg of steroids per 100 ml of the diet. The vitamins were added as 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 per cent and the amino acids at 0.1 per cent. Crude leaf extracts of four different plants at the concentration of 5 and 10% were also incorporated in the diet composition. All the ingredients mentioned above were added

separately at the end of the diet preparation after addition of agar-agar and cooling the diet to about 20 °C. After the preparation of the diet, the larvae at late third instar stage were released and were evaluated for the growth parameters.

For the estimation of protein and lipid content, the following concentrations were selected based on the superior larval growth parameters which were recorded in the above experiments conducted. In the case of steroids, 0.01% was selected as it is deleterious to the larval growth, to know their influence of protein and lipid contents of the larvae.

Steroids		Source
1.	Betamethasone 0.002 & 0.01%	Glaxo India Ltd., Nashik
2.	Dexametasone 0.002 & 0.01%	Fulford India Ltd., Mumbai
3.	Stonazolol 0.004 & 0.01%	Cadila Health Care, Ahmadabad
4.	Prednisolone 0.008 & 0.01%	Wyeth Lederle Ltd., Mumbai
5.	Durabolin 0.002%	Infar India Ltd., Culcutta
Vitamins		
1.	Vitamin B 0.1%	E. Merch (India) Ltd., Goa
2.	Vitamin B with C 0.25%	E. Merch (India) Ltd., Goa
3.	Vitamin C 0.5%	Glaxo India Ltd., Nashik
1.	Vitamin E 0.05%	E. Merch (India) Ltd., Goa
Aminoacids		Plant extracts
1.	Tryptophan 0.1%	<i>Tribulus terrestris</i> 10%
2.	Methionine 0.1%	<i>Ocimum terrestris</i> 10%
3.	Arginine 0.1%	<i>Eucalyptus naundia</i> 10%
4.	Threonine 0.1%	<i>Datura stramonium</i> 10%
Oils		
1.	Groundnut oil 1%	
2.	Soybean oil 1%	
3.	Castor oil 1%	
4.	Sunflower oil 1%	

The diets were prepared by adding the above mentioned ingredients separately and were maintained as different treatments. For the estimation of lipid content, the cholesterol was not added in the diets which were maintained as treatments (i.e. those which were prepared with steroids, vitamins, amino acids, plant extracts and oils), and the basic diet with cholesterol served as control. After the preparation of the diet, late third instar larvae were released. Four replications were maintained with ten larvae per replication, Four, fourth instar larvae were individually analysed for each replication and the larval weight was recorded before analysing the larvae.

#### *Estimation of total protein content*

The estimation was carried out as per the method of Lowry *et al.* (1951). A standard curve was prepared using Bovine Serum Albumin (BSA). Then the samples were incubated at room temperature overnight for the color development and the absorbance was read at 660 nm in a spectrophotometer (Spectronic –20<sup>R</sup>). The total protein content was expressed in mg per gram of larvae.

### *Estimation of total lipid content*

Fourth instar larvae from the respective treatments were dissected and haemolymph was collected and the samples were pooled to make up the volume of haemolymph from each treatment as 2 ml.

### *Lipid extraction*

Lipids were extracted from pooled haemolymph samples (2.0 ml) by standing with 40 ml chloroform - methanol (2 : 1) overnight, in darkness and at room temperature (Folch *et al.*, 1951). All operations were carried out under nitrogen when possible, all glasswares and filter papers were rinsed with hot methanol.

The extraction mixture was filtered using Whatman No. 1 filter paper and the residue was washed with extraction solvent. Combined washes and filtrate were washed with water (0.2 vol) to remove non-lipid impurities and then dried and weighted as previously outlined (Bennett and Shotwell, 1970).

### *Lipid estimation*

This was done through Silicic acid column chromatography. Partially purified lipids were suspended in a minimal volume of anhydrous diethyl ether-hexane (1 : 1) and quantitatively transferred to a 1 g silicic acid column. Columns were poured as silicic acid ether slurries and washed by gravity flow overnight with anhydrous diethyl ether. After sample application, the columns were operated under nitrogen pressure (90 mm Hg). Neutral lipids were eluted with anhydrous diethyl ether (5 ml) and phospholipids were then eluted with spectroquality methanol (5 ml). After neutral lipid and phospholipid fractions were evaporated under nitrogen and desiccated with phosphorus pentoxide, dry weights were determined. The total lipid content expressed in mg/ml of larval haemolymph.

## RESULTS AND DISCUSSION

### **Total protein**

Estimated total protein content was found to be maximum (2.5 mg/g) in larvae reared on diets with stonzolol 0.004 per cent and the minimum (0.963 mg/g) in durabolin 0.002 per cent (Table 1), since the former might have interacted favourably and the latter adversely. The least content was 2.6 folds lesser than the highest content recorded. The stonzolol treatment was followed by prednisolone 0.008 per cent where the total protein content was 2.280 mg/g. The other treatments were on par with control. The steroids, all recorded with least content of protein at 0.01 per cent showed an adverse effect on larval metabolism. Thus it was found that generally the protein content was more when the larval weight increased (Fig. 1).

The vitamins, altered by total protein content of larvae and it was more in diets added with vitamin C 0.5 per cent (1.950 mg/g), tryptophan 0.1 per cent (1.875 mg/g) and *T. terrestris* 0.1 per cent (1.5 mg/g). The treatments with vitamin C was followed

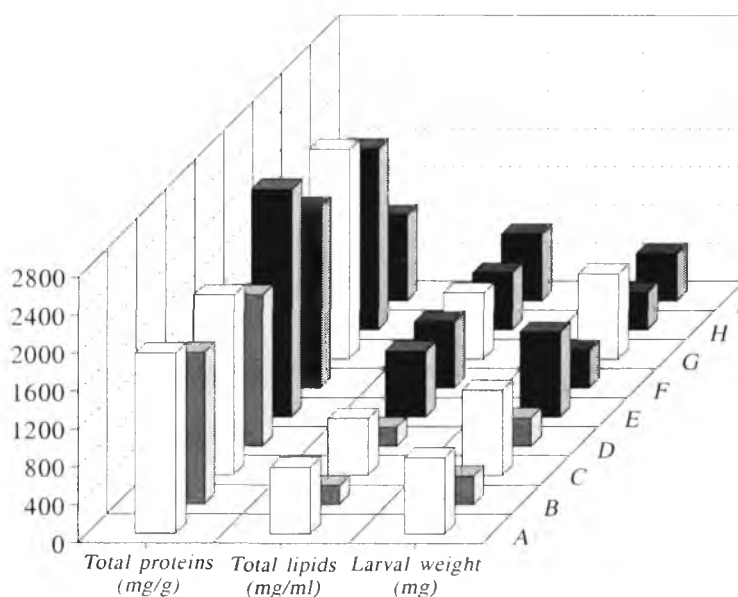


FIGURE 1. Influence of steroids of larval weight, total protein and lipid content of *S. litura*. A: Betamethasone 0.002%, B: Betamethasone 0.01%, C: Dexamethasone 0.002%, D: Dexamethasone 0.01%, E: Stonzolol 0.004%, F: Stonzolol 0.01%, G: Prednisolone 0.008%, H: Prednisolone 0.01%, I: Durabolin 0.002%.

TABLE 1. Protein and lipid profile in *S. litura* larvae fed on semi-synthetic diets with different steroids

Treatments	Total protein content (mg/g) @	Total lipid content (ug/ml) @
Expt 1		
1. Betamethasone 0.02%	2.017 <sup>abc</sup>	717.00 <sup>abc</sup>
2. Betamethasone 0.01%	1.707 <sup>abc</sup>	217.00 <sup>d</sup>
3. Dexamethasone 0.002%	2.017 <sup>abc</sup>	717.00 <sup>abc</sup>
4. Dexamethasone 0.01%	1.710 <sup>abc</sup>	200.00 <sup>d</sup>
5. Stonzolol 0.004%	2.500 <sup>a</sup>	738.00 <sup>ab</sup>
6. Stonzolol 0.01%	1.920 <sup>abc</sup>	625.30 <sup>abc</sup>
7. Prednisolone 0.008%	2.280 <sup>ab</sup>	726.00 <sup>abc</sup>
8. Prednisolone 0.01%	1.907 <sup>abc</sup>	624.50 <sup>abc</sup>
9. Durabolin 0.002%	0.963 <sup>c</sup>	596.75 <sup>c</sup>
10. Control	1.667 <sup>abc</sup>	750.00 <sup>a</sup>

@ - Mean of 3 observations. In a column means followed by similar letters are not statistically different ( $P = 0.05$ ) by DMRT.

TABLE 2. Protein and lipid profile in *S. litura* larvae fed on semi-synthetic diets with different vitamins and aminoacids

Treatments	Total protein content (mg/g) @	Total lipid content (ug/ml) @
Expt 2		
1. Vitamin B 0.1%	1.513 <sup>bc</sup>	610.25 <sup>b</sup>
2. Vitamin B and C 0.25%	1.710 <sup>ab</sup>	610.75 <sup>b</sup>
3. Vitamin C 0.5%	1.950 <sup>a</sup>	650.50 <sup>b</sup>
4. Vitamin E 0.05%	1.440 <sup>bc</sup>	606.75 <sup>b</sup>
5. Control	1.250 <sup>c</sup>	700.00 <sup>a</sup>
Expt 3		
1. Tryptophan 0.1%	1.875 <sup>a</sup>	348.80 <sup>b</sup>
2. Methionine 0.1%	1.430 <sup>b</sup>	347.20 <sup>b</sup>
3. Arginine 0.1%	1.283 <sup>b</sup>	235.40 <sup>c</sup>
4. Threonine 0.1%	1.513 <sup>b</sup>	235.60 <sup>c</sup>
5. Control	1.250 <sup>b</sup>	700.00 <sup>a</sup>

@ - Mean of 4 observations. In a column means followed by similar letters are not statistically different ( $P = 0.05$ ) by DMRT.

by vitamin B with C and other two treatments (vitamin B and vitamin E) were on par and significantly different from control. Tryptophan was found to be superior statistically to other treatments (Table 2). In case of plant extracts, the protein content was indicated as the least in *D. stramonium* (0.780 mg/g) and other treatments were on par with control (Table 3). The increased larval weight obtained through the favourable effects by vitamin C, tryptophan and *T. terrestris* on larval metabolism led to the increased protein content. Zeenath and Nair (1994) found that when the JHA (hydroprene) was treated, the larvae showed accumulation of haemolymph protein and an increased in total aminoacid concentration in the haemolymph. In the present studies, stonzolol (0.004%), Vitamin C (0.5%), tryptophan (0.1%) and *T. terrestris* (10%) showed positive influence on protein metabolism. The addition of oils did not show any influence on protein content of the larvae.

### Total lipid

Based on observations on lipid content estimated using larval samples from different diets, it was found that the treatments with steroids were on par with control. The control recorded a lipid content of 750 mg/ml which was followed by diet with stonzolol 0.004 per cent (738.0 mg/mg).

The least content of 217 mg/ml and 200 mg/ml were noticed in larvae reared on diets with betamethasone 0.01 per cent and dexamethasone 0.01 per cent (Table 1). The trend was the same when the addition of vitamins, aminoacids and plant extracts (Tables 2 and 3) with diet was done. In the case of diets with oils also, the treatments were not significantly different from control. Comparison and characterization of lipids extracted from haemolymph of healthy and diseased larvae (5.7 to 7.2 mg/ml)

TABLE 3. Protein and lipid profile in *S. litura* larvae fed on semi-synthetic diets with different plant extracts and oils

Treatments	Total protein content (mg/g) @	Total lipid content (ug/ml) @
Expt 4		
1. <i>T. terrestris</i> 10%	1.500 <sup>a</sup>	250.00 <sup>b</sup>
2. <i>O. sanctum</i> 10%	1.368 <sup>ab</sup>	280.00 <sup>b</sup>
3. <i>E. naundia</i> 10%	1.200 <sup>ab</sup>	268.00 <sup>b</sup>
4. <i>D. stramonium</i> 10%	0.780 <sup>b</sup>	208.00 <sup>b</sup>
5. Control	1.210 <sup>ab</sup>	750.00 <sup>a</sup>
Expt 3		
1. Groundnut oil 1%	1.152	725.00
2. Soybean oil 1%	1.026	710.00
3. Castor oil 1%	1.024	710.00
4. Sunflower oil 1%	1.210	740.00
5. Control	1.210	750.00
	NS	NS

NS - Not significant. @ - Mean of 4 observations. In a column means followed by similar letters are not statistically different ( $P = 0.05$ ) by DMRT.

and was reduced to 3.1 to 3.9 mg/ml in diseased larvae (Bennett and Shotwell, 1970). The protein and lipid content decreased when larvae of *Dicladispa armigera* were treated with the JHA, methoprene (Baishaya and Hazarika, 1996).

The favourable effect in maintaining the lipid content same as that of cholesterol in control by the addition of steroids and oils was shown in the present investigation.

Thus it was clear that the diets with stonzolol, vitamin C, tryptophan and *T. terrestris* at specified concentrations showed favourable effect on the larval growth in terms of protein content as compared to the control. So, these can be incorporated into the basic diet composition. The steroids and oils at specified concentrations recorded with the lipid content same as that of control with cholesterol and thus, the cholesterol in the basic diet can be replaced by them.

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## Hyperglycaemic Response of Fat Body from *Spodoptera mauritia* (Noctuidae: Lepidoptera) to Neuropeptide Hormones

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**ABSTRACT:** The effect of neurohormones extracted from the neuronal tissues of *Spodoptera mauritia* and synthetic locust adipokinetic hormone-I (syn Lom AKH-I) on sugar release from the fat body of *S. mauritia* has been studied *in vitro*. The hormones elicited hyperglycaemia hypoglycaemia to various extents in the fat body of the stages tested. There seemed to have no defined dose-response. The hyperglycaemic effect was more pronounced in the adult stage. In adults, synthetic AKH showed stimulatory activity at all the concentrations (0.02, 0.05, 0.1, 0.25 and 0.5  $\mu\text{M}$ ) except the highest (1.0  $\mu\text{M}$ ). The maximum stimulation of sugar release (230 mg/g fat body) was obtained with a concentration of 0.05  $\mu\text{M}$  of the synthetic hormone. The maximum stimulation obtained with the hormone extract (0.01 gpe) was only 47 mg/g fat body which was only 20% of the minimum stimulation obtained with the syn AKH. Hyperglycaemic effect on larval, prepupal and pupal fat body were not appreciable. In these stages, stimulation of sugar release were 25 mg/g fat body. In the adult fat body, the optimum concentration of the hormones for maximum release were 0.01 gpe and 0.05  $\mu\text{M}$  respectively. The highest concentration of both the hormones were found to be inhibitory in activity. The overall activity of the synthetic hormone was much better than that of the extract. This is presumed to be due to the presence of counteracting components in the crude extract of the neuronal tissues.

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**KEYWORDS:** *Spodoptera mauritia*, adipokinetic hormone, hyperglycaemic hormone, hypoglycaemic hormone, cc-extract.

### INTRODUCTION

Carbohydrates together with proteins and lipids are the principal class of organic compounds found in insects as well as other organisms. In most insects carbohydrate reserves are present as glycogen and trehalose, which can be readily converted into glucose (Islam and Roy, 1981). Carbohydrates provide instant energy for activities such as flight in most of the insects. Trehalose, which is a non-reducing disaccharide,

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found in the haemolymph as the circulating sugar in insects, also functions as an important reserve carbohydrate in them (Bloemen *et al.*, 1987; Lee and Keeley, 1994; Siegert, 1995; Becker *et al.*, 1998). Haemolymph trehalose level is maintained by the activity of phosphorylase whose activity is regulated by hormone-induced cascade of reactions. Various hormones have been found to be involved in the regulation of carbohydrate metabolism. Hormonal control of carbohydrate metabolism has been studied in a large number of insects (Gaede and Lohr, 1982; Ziegler and Schulz, 1986; Wilps and Gaede, 1990; Raina *et al.*, 1995; Keeley *et al.*, 1996; Becker *et al.*, 1998). The lipid and trehalose mobilising hormones in insects are secreted by the corpus cardiacum and corpus allatum complex and belong to a family of peptides (AKH/RPCH) (Gaede, 1990). The characteristic activity of these peptides in insects is to regulate energy metabolism by mobilising fat body nutrient reserves into circulating haemolymph (Gaede, 1990). Some of these hormones are now known to be multifunctional. The locust adipokinetic hormone, for example, have other functions such as regulation of fatty acid oxidation in flight muscles (Robinson and Goldsworthy, 1977), inhibition of protein synthesis (Carlisle and Loughton, 1986), cardio-acceleration (Scarborough *et al.*, 1984), myoactivity (O'Shea *et al.*, 1984), interconversion of lipoproteins in haemolymph (Wheeler and Goldsworthy, 1985), and inhibition of fatty acid synthesis (Gokuldas *et al.*, 1988). At present more than 30 peptide hormones from different insect orders have been included in this family (Gaede, 1990, 1992; Keeley *et al.*, 1991) of peptides.

Corpora cardiaca extract when injected into the haemolymph was found to elevate the level of glycogen phosphorylase (Steele, 1963) and haemolymph carbohydrates (Goldsworthy *et al.*, 1972) in *P. americana*. Goldsworthy (1970) demonstrated a decrease in locust fat body phosphorylase activity following cardiectomy. Injection of locust CC-extract which causes elevation in the haemolymph lipid in the locusts (Goldsworthy *et al.*, 1972), on the other hand, has been shown to induce hyperglycaemia in cockroaches (Holwerda *et al.*, 1977). Such cross-reactivity studies have also been carried out in the phasmid *Sipylodea sipylus*, the CC-extract of which elevated the levels of haemolymph lipids in locusts and the levels of blood carbohydrates in cockroaches (Gaede, 1984).

The present study investigates the effect of native neurohormones extracted from the neuronal tissues (brain-corpora cardiaca-corpora allata complex) and synthetic locust AKH-I on sugar release *in vitro* from the fat body of the paddy armyworm *Spodoptera mauritia* during development.

## MATERIALS AND METHODS

### Insects

A colony of the paddy armyworm moth, *S. mauritia* has been maintained in the laboratory on the grass *Ischaemum aristatum* (larvae) and diluted honey (adults). Stages used for the present study were 96 h sixth instar larva, prepupa, pupa and adult.

**Synthetic locust adipokinetic hormone-I (Syn Lom-AKH-I)**

This was obtained from Peninsula Laboratories, USA. A stock hormone solution of 200 pmoles/ $\mu$ l was prepared in glass-distilled water from which different concentrations of the hormone (0.02, 0.05, 0.1, 0.25, 0.5 and 1.0  $\mu$ M) were prepared by appropriate dilution.

**Physiological saline (pH 7.2)**

The buffer used for fat body incubation contained NaCl, 154 mM; KCl, 8.0 mM;  $\text{CaCl}_2$ , 1.8 mM and HEPES, 30 mM.

**Preparation of *Spodoptera* hormone extract (SH-extract)**

The brain along with corpora cardiaca and corpora allata complex were removed from many adult insects and collected in distilled water (5  $\mu$ l per gland complex) taken in an ependorf tube and the hormone was extracted by heating the tube in a steam bath for 5 minutes. The tissue was separated by centrifuging at 5000 g in a microfuge. The clear supernatant was used as the *Spodoptera* hormone extract (SH-extract). Lower concentrations of the extract (0.001, 0.01, 0.1 and 1.0 gpe) were prepared by dilution with distilled water. Concentration is expressed as gland pair equivalent (gpe).

***In vitro* incubations**

For *in vitro* incubations, fat body from individual insects were removed, washed in saline and blotted, chopped, mixed and divided into two halves. One half served as experimental and the other half as control. These halves were then put into preweighed incubation vials (flat bottomed, 5.0 ml capacity glass tubes with bakelite screw caps) containing 200  $\mu$ l incubation buffer and 10  $\mu$ l of either the hormone of appropriate concentration (experimental) or 10  $\mu$ l of distilled water (control) and the fat body weights were determined. Incubations were carried out for 30 min in a shaker water bath set at 37 °C.

**Measurement of sugar release**

After the *in vitro* incubation, samples of the incubation medium from both the controls and experimentals were drawn and the amount of sugar present in the samples were measured colorimetrically using anthrone method (Mokrasch, 1954) with threhalose as standard.

**Analysis of data**

The values obtained were made homoscedastic by appropriate transformation and subjected to ANOVA by methods described by Rao (1965) and Das and Giri (1986). Significance of the difference in activity between different stages, between different concentrations and the combined effects of both the stages and the concentrations were tested in the controls and experimentals and the values obtained as their difference were analysed separately (Tables 1 and 2).

TABLE 1. ANOVA of data for sugar release from the fat body of various stages of *S. mauritia* at different concentrations of SH-extract *in vitro*

Source	df	SS	MSS	F	Tab. F
Stages (S)	3	8.925314	2.975105	2.60	2.61
Conc. (C)	3	19.13079	6.376932	5.58	2.10
S $\times$ C	9	12.68929	1.409921	1.23	1.57
Error	112	127.9999	1.1429857		

Data used were difference between experimentals and controls from 128 cases. The table F-value is taken at 5% level of significance. Values used were transformed to make them homoscedastic (see Materials and Methods section).

TABLE 2. ANOVA of data for sugar release from the fat body of various stages of *S. mauritia* at different concentrations of syn AKH *in vitro*

Source	df	SS	MSS	F	Tab. F
Stages (S)	3	9.785206	3.261735	2.85	2.61
Conc. (C)	5	46.80383	9.360765	8.19	2.10
S $\times$ C	15	48.25604	3.217069	2.18	1.57
Error	168	192	1.142857		

Data used were difference between experimentals and controls from 192 cases. The table F-value is taken at 5% level of significance. Values used were transformed to make them homoscedastic (see Materials and Methods section).

## RESULTS

### Hyperglycaemic activity of SH-extract *in vitro* on *S. mauritia* fat body

Fat body from 96 h larva, prepupa, pupa and adult of *S. mauritia* were incubated for 30 minutes with different concentrations of SH-extract and quantitative changes in total sugar released over control were measured. Figure 1 shows the pattern of activity of SH-extract on sugar release from the fat body of different stages of *S. mauritia*. It appears that the hormone had a stimulatory effect on fat body sugar release in most of the cases but this effect was not dose-dependent in any of the stages. In 96 h larva, stimulation was shown by 0.1 gpe concentration (4 mg/g fat body). All the other concentrations showed negative effect on sugar release. In prepupal fat body maximum amount of sugar was released (17 mg/g fat body) with 0.1 gpe. The highest concentration (1.0 gpe) also showed stimulation (8 mg/g fat body) whereas other concentrations showed inhibition. Pupal fat body showed good stimulatory response. Maximum release of sugar occurred with the lowest concentration 0.001 gpe (23 mg/g fat body) followed by 0.1 gpe (20 mg/g fat body). In the case of adult, stimulation of sugar release was maximum with a hormone concentration of 0.01 gpe. There was an increase in sugar by about 47 mg/g fat body. The lowest concentration (0.001 gpe) gave inhibition of 3 mg/g fat body. It was seen that the different hormone

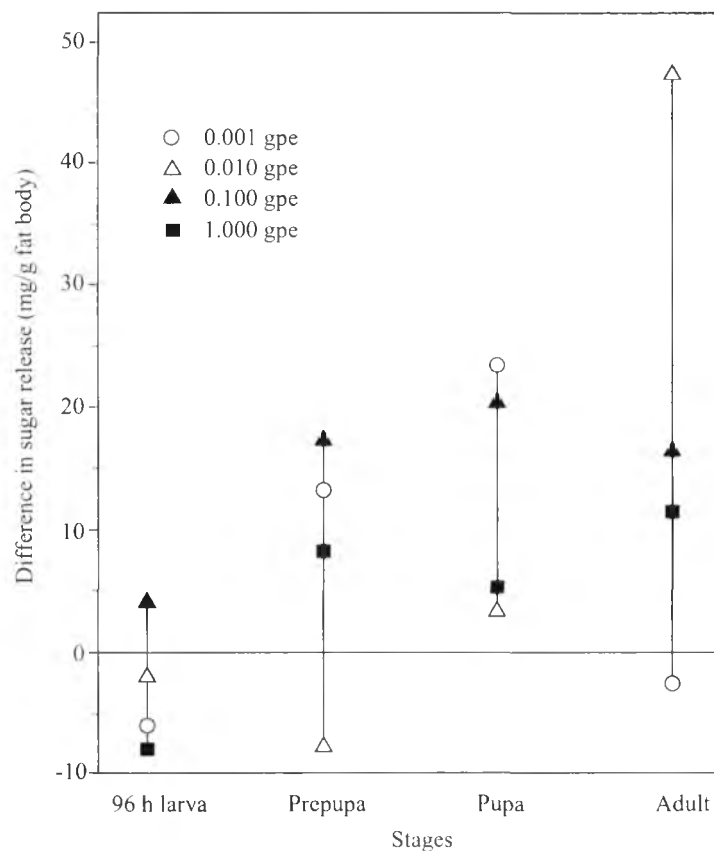


FIGURE 1. Effect of different concentrations of SH-extract on sugar release *in vitro* from the fat body of various stages of *S. mauritia*.

concentrations had different pattern of activity with respect to sugar release. Better stimulatory activity was shown by lower concentrations than higher concentrations. Figure 2 provides the average sugar release from the fat body of *S. mauritia* in the presence of different hormone concentrations (experimentals). Maximum amount of sugar was released by the adult fat body (around 130 mg/g fat body) except with the lowest concentration (0.001 gpe). In 96 h larval fat body, sugar release with different concentrations ranged between 57 mg/g fat body and 36 mg/g fat body. In prepupa maximum amount of sugar released was 76 mg/g fat body with a concentration of 0.1 gpe. Pupal fat body showed good hyperglycaemic response to 0.001 gpe concentration (109 mg/g fat body) but not to other concentrations.

The values obtained (difference between the experimental and the controls) were subjected to ANOVA and is presented in Table 1. The results show that the effect of SH-extract between different stages and the combined effect of stages and concentrations

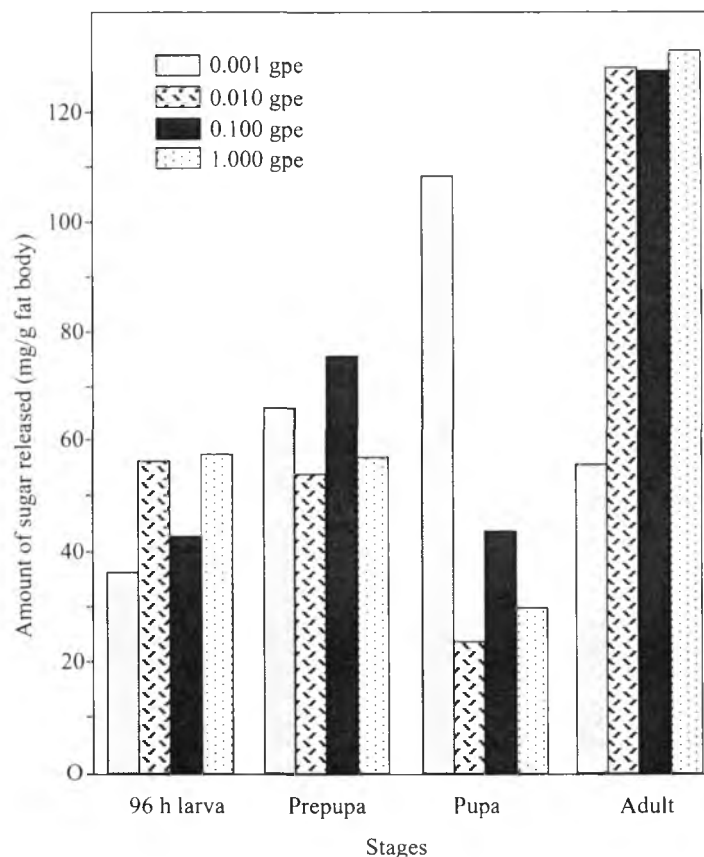


FIGURE 2. Average sugar release from the fat body of *S. mauritia* incubated with different concentrations of SH-extract in various stages.

on fat body sugar release were not statistically significant whereas the effect between the different concentrations were significant.

#### Hyperglycaemic activity of syn locust AKH-1 *in vitro* on *S. mauritia* fat body

Fat body from 96 h larva, prepupa, pupa and adult of *S. mauritia* when tested for hyperglycaemic response to different concentrations of syn AKH showed that syn AKH have significant effect on sugar release from the fat body. Figure 3 presents the effect of AKH on sugar release from the fat body of the various stages of *S. mauritia*. In 96 h larval fat body, the hormone at all the concentrations tested except  $1.0 \mu\text{M}$ , showed hyperglycaemic effect of various degrees. The maximum stimulatory effect on fat body sugar release in this case (by about  $19 \text{ mg/g fat body}$ ), was given by  $0.25 \mu\text{M}$ . In the case of prepupal fat body, the hormone had relatively very small effect on sugar release except  $0.25 \mu\text{M}$ , which showed some inhibitory effect. In the pupal

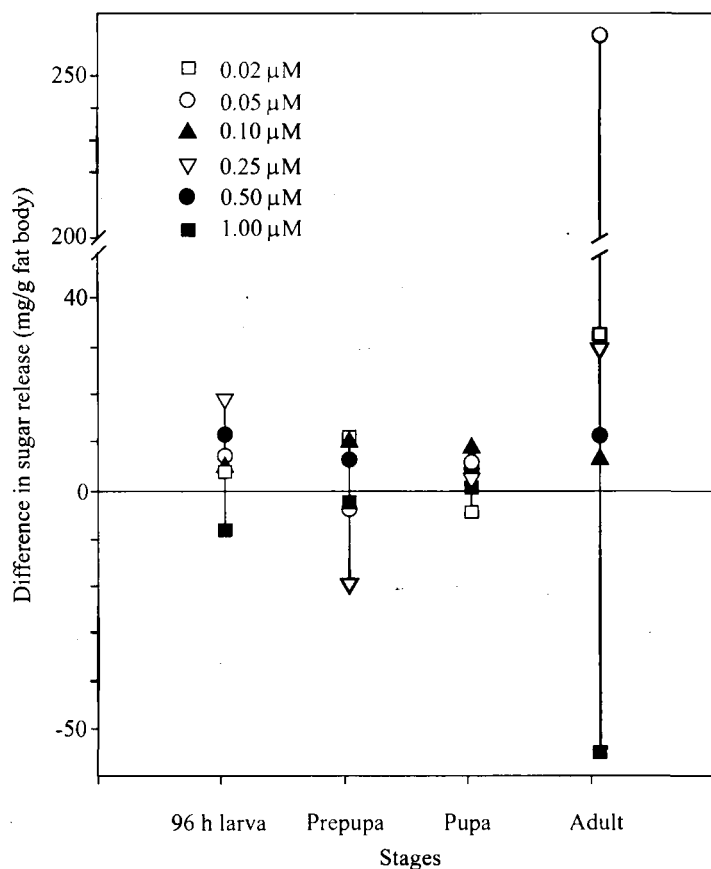


FIGURE 3. Effect of different concentrations of syn AKH-I on sugar release *in vitro* from the fat body of various stages of *S. mauritia*.

stage also, the effect was negligible. With adult fat body, a hormone concentration of  $0.05 \mu\text{M}$  showed very high stimulatory effect (by about  $264 \text{ mg/g fat body}$ ). However, the highest concentration ( $1.0 \mu\text{M}$ ) showed an inhibitory effect ( $50 \text{ mg/g fat body}$ ) while the rest of the concentrations showed moderate stimulatory effects. Figure 4 presents the average release of sugar from the fat body of *S. mauritia* in the experimental observations. The adult fat body showed very high sensitivity to hyperglycaemic factors (sugar release  $600 \text{ mg/g fat body}$ ) compared to all the other stages where the sugar release was below and around  $100 \text{ mg/g fat body}$ . However this effect was seen only with a concentration of  $0.05 \mu\text{M}$ . With other concentrations the release were only around  $100 \text{ mg/g fat body}$  which were still better than the hyperglycaemic activity seen in other stages.

The values for the difference between experimentals and controls were subjected to ANOVA and is presented in Table 2. The F-values in all the three cases (between

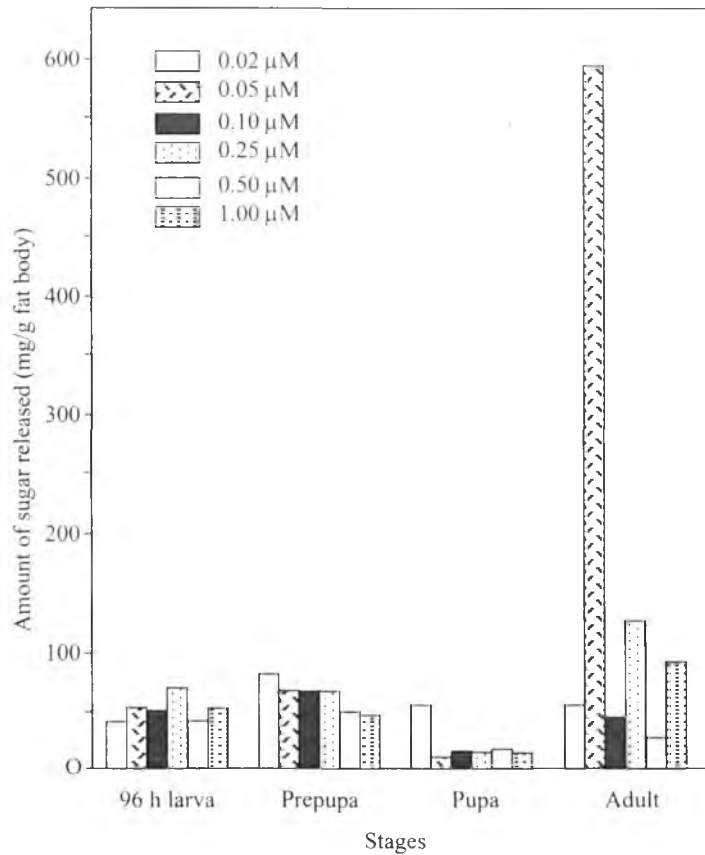


FIGURE 4. Average sugar release from the fat body of *S. mauritia* incubated with different concentrations of syn AKH in various stages.

stages, between concentrations and between the combined effects of stages and concentrations) were greater than the Table F-values showing that the effects are significant.

#### DISCUSSION

The presence of hyperglycaemic hormones (HGHs) involved in the regulation of carbohydrate metabolism have been reported in many insects (Steele, 1961; Ziegler, 1979; Witten *et al.*, 1984; Gaede and Rinehart, 1987; Gaede *et al.*, 1988). The HGHs were first reported in the cockroach, *P. americana* (Steele, 1961). The HGHs are peptide hormones that activate glycogen phosphorylase in the animal's metabolic tissue to convert glycogen reserve into circulating carbohydrates as an energy source (Steele and Hall, 1985). Locusts utilise fat body glycogen as an important fuel for initial stages of flight (Beenackers *et al.*, 1984). During flight AKH is released from the

CC into the haemolymph (Goldsworthy and Wheeler, 1984) and induces the activation of phosphorylase in the fat body (Van Marrewijk *et al.*, 1985).

In our experiment, it was observed that syn AKH-I and the native hormone extract (SH-extract) had stimulatory effect on the sugar release from the fat body of *S. mauritia* *in vitro*, although the activity of the latter was statistically not significant between different stages tested. Activity was found to be prominent in the adult compared to other stages, although this was not true with all the tested concentrations. In the adult, AKH at the highest concentration ( $1.0 \mu\text{M}$ ) showed inhibitory activity whereas all other concentrations showed stimulation to various extents. SH-extract on the other hand, elicited hyperglycaemia to various extents with all the stages, but not with all the concentrations. Some concentrations showed inhibitory activities also. In the case of AKH, the maximum stimulation obtained (with a concentration of  $0.05 \mu\text{M}$ ) with the adult fat body (264 mg/g fat body) was much greater than the stimulation obtained in other stages, although all these were statistically significant. Maximum stimulation obtained with SH-extract on adult fat body (47 mg/g fat body) on the other hand, was not considerably higher than the stimulation shown by other stages and concentrations. In the case of AKH, all stages except adult showed only moderate response with all the concentrations of the hormone, the effect being smaller compared to that found in the adult fat body. Stimulation and inhibition of sugar release by SH-extract and AKH ranged within about 50 mg/g fat body except a single case (about 260 mg/g fat body with  $0.05 \mu\text{M}$  AKH in the adult fat body) (Fig. 3) which appears to be an extremely high value compared to other values obtained. The corresponding total lipid release value for this particular case also was very high (600 mg/g fat body) (Fig. 4). This, most likely, may be due to the difference in the metabolic status of the fat body from different batches of insects used.

From the results obtained it appears that SH-extract contained compounds which are able to elicit hyperglycaemia in all the stages. However, the degrees of response of the fat body to the hyperglycaemic factors differed in different stages of development. Larval fat body seemed to be the least responsive and adult fat body showed the maximum response. AKH also elicited response, which is similar to that of the SH-extract suggesting the similarity of the active component in the SH-extract to AKH-I. However, in both the cases, there seemed to have no dose-response. Stimulatory effect was more convincing in the case of AKH. The extract on the other hand showed hyperglycaemic effect as well as hypoglycaemic effect, more so in the larval fat body. This dual effect may be due to the co-existence of hyper- and hypoglycaemic factors, which is useful for the insect to regulate the direction of movement of metabolites as per requirement. Larval stages are meant mostly for eating and storing reserve materials for use during pupal stage for the development of adult structures. Therefore, at this stage the presence of a hypoglycaemic hormone is justified as hypoglycaemic hormone will not only preserve glycogen in the fat body but also will promote protein synthesis as reported in the case of insulin in vertebrates (Marcus and Raghavan, 1999). Hypoglycaemic or insulin like factors have been described from many insects though they are not proved to be exclusively involved in the

regulation of sugar metabolism. They may have different physiological roles (Tager *et al.*, 1976; Duve *et al.*, 1979; Marcus and Raghavan, 1999). Certain hormones are active at lower concentrations and without activity at higher concentrations. A hormone with different activities at different concentrations is exemplified by locust AKH. At lower concentrations, which is insufficient to elicit adipokinetic effect, the hormone inhibited protein synthesis (Carlisle and Loughton, 1986) whereas higher concentrations stimulates lipid release. A better hyperglycaemic activity found in pupal fat body could possibly be an indication to the gradual activation of the system during development inside the pupa, which should be there in the adult fat body. The pupae selected for the study were mostly 6 days old, which means the fat body almost have completed the reorganisation of the larval fat body into adult fat body. Since the adult *Spodoptera* has a very brief life span (just about 5 days) unlike insects such as locusts, not much of the consumed sugary food (nectar or honey) is converted into lipids for storage. Therefore it is reasonable to believe that the adult insects might be having carbohydrate based metabolic activities. Such a discriminative type of sensitivity to hormones is found in other insects such as *M. sexta* where the fat body exhibits stage-specific sensitivity to AKH peptides. The peptides regulate mobilisation of carbohydrates in larvae and lipids in adults (Ziegler *et al.*, 1984; Ziegler, 1990).

Cross-reactivity of syn AKH-I in *S. mauritia* indicates the presence of similar active groups in the hormone molecules. The hyperglycaemic activity of syn AKH-I in *S. mauritia* thus lends support to the possibility that the active component of SH-extract may be a peptide having structure similar to that of syn AKH-I. However, separation and purification of the active components of SH-extract using RP-HPLC only will reveal such details.

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## Five New Species of Predatory Mites (Acarina: Phytoseiidae) from Kerala, India

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**ABSTRACT:** A study was conducted on the biodiversity of predatory acarines in different agro ecosystems of various districts in Kerala state. The study revealed the occurrence of many species of predatory fauna under the family Phytoseiidae inhabiting, different flora under varied ecological conditions. Out of these, five new species viz., *Amblyseius apocynae* sp. nov. *A. pseudorientalis* sp. nov. *A. rubiae* sp. nov. *A. apocynaevagrans* sp. nov. and *A. keralensis* sp. nov. are described.

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**KEYWORDS:** acarina, *Amblyseius*, predatory mites, new records.

### INTRODUCTION

Predacious mites of the family Phytoseiidae have been recognized as one of the most valuable groups of predators on plant feeding mites since the beginning of this century (Chant, 1959). Mass multiplication methods have been developed to use these predators in commercial scale on a variety of crops. (Lo *et al.*, 1979; Krishnamoorthy, 1982) some of the indigenous species have been identified and reared in laboratory through very simple methods (Krishnamoorthy, 1990). More than two hundred species have been described under predatory families from different agroecological conditions in India (Gupta, 1985). However, the reports on occurrence of new species are scanty from peninsular India. Hence a systematic survey was undertaken to study the occurrence of new species of Phytoseiidae from certain districts viz., Kottayam, Pathananthitta, Thiruvananthapuram, Palakkad and Trichur of Kerala state. The study revealed a rich diversity of phytoseiid fauna from various flora in these regions. All these five new species have been described with enough illustrations in this paper for the first time from Kerala State.

\*Corresponding author

## MATERIALS AND METHODS

Frequent visits were undertaken to collect the mites from different study locations. Mites of comparatively larger size were collected directly from the foliage using a handlens and a camel hairbrush and preserved in ethyl alcohol until permanent slides were prepared. Apart from this, leaves and twigs were collected in polythene bags and examined under zoom microscope to collect the mites. Preparation of permanent slides were made in Hoyer's medium. The specimens were thoroughly examined and identified with the help of available literature. Measurements are given in microns. All the Holotypes and paratype slides have been deposited in the acarological collections maintained at the Department of Entomology, TNAU, Coimbatore 3.

The following abbreviations have been used to denote the various structures and parts: DF - Dorsum; of ♀ VF - Ventrum of ♀, VM - Ventrum of Male, SP - Spermatheca, MP - Metapodal plates, PB - peritremal plates Cf - Chelicera of female, VASf - Ventri Anal Shield of Female, VASm - Ventri Anal Shield of Male Chelicera of male - LI-LIV-Legs I to IV.

## Description of species

1. *Amblyseius (Amblyseius) apocynae* sp. nov. [Figs 1 and 1(a)]*Female*

Dorsal shield smooth, with 17 pairs of setae, 315 long, 221 wide. Setae V,  $L_1$ ,  $L_4$ ,  $M_3$  and  $L_8$  long, measuring 30, 50, 82-86, 96 and 210 respectively. Other dorsal setae minute. Two pairs of sublateral setae on lateral integument measuring  $S_1$ -12, and  $S_2$ -6. More than 6 pairs of prominent pores present. Sternal shield wider (80) than long (60) with three pairs of sternal setae (30) and 2 pairs of visible pores, 4th pair on the metasternal platelets, which are well sclerotized and 12 long. Genital shield 80 wide with a pair of genital setae. A thin folding also present in the interspace. The ventrianal shield pentagonal, slightly tapering towards posterior end, measuring 110 long and 84 wide with 3 pairs of preanal setae and a pair of semi circular pores; 4 pairs of setae present on integument around the ventrianal shield,  $VL_1$  measuring 82 long. Spermatheca with very short cervix (14). Two pairs of metapodal platelets present, primary one 20 long, and secondary one 10 long.

*Gnathosoma (cf)*

Fixed digit of chelicera multidentate while movable digit with 3-5 fine teeth.

*Legs (LI-IV)*

Macrosetae present on genu I, genu II, genu III, tibia III, basitarsus III, genu IV, tibia IV and basitarsus IV, measuring 30, 38, 52, 38, 24, 104, 62 and 60 long respectively. The chaetotactic formula:

$$\text{genu II, } \begin{array}{cc} 2 & 1 \\ 1 & 0 \end{array} 1; \quad \text{tibia II, } \begin{array}{cc} 2 & 1 \\ 1 & 1 \end{array} 1;$$

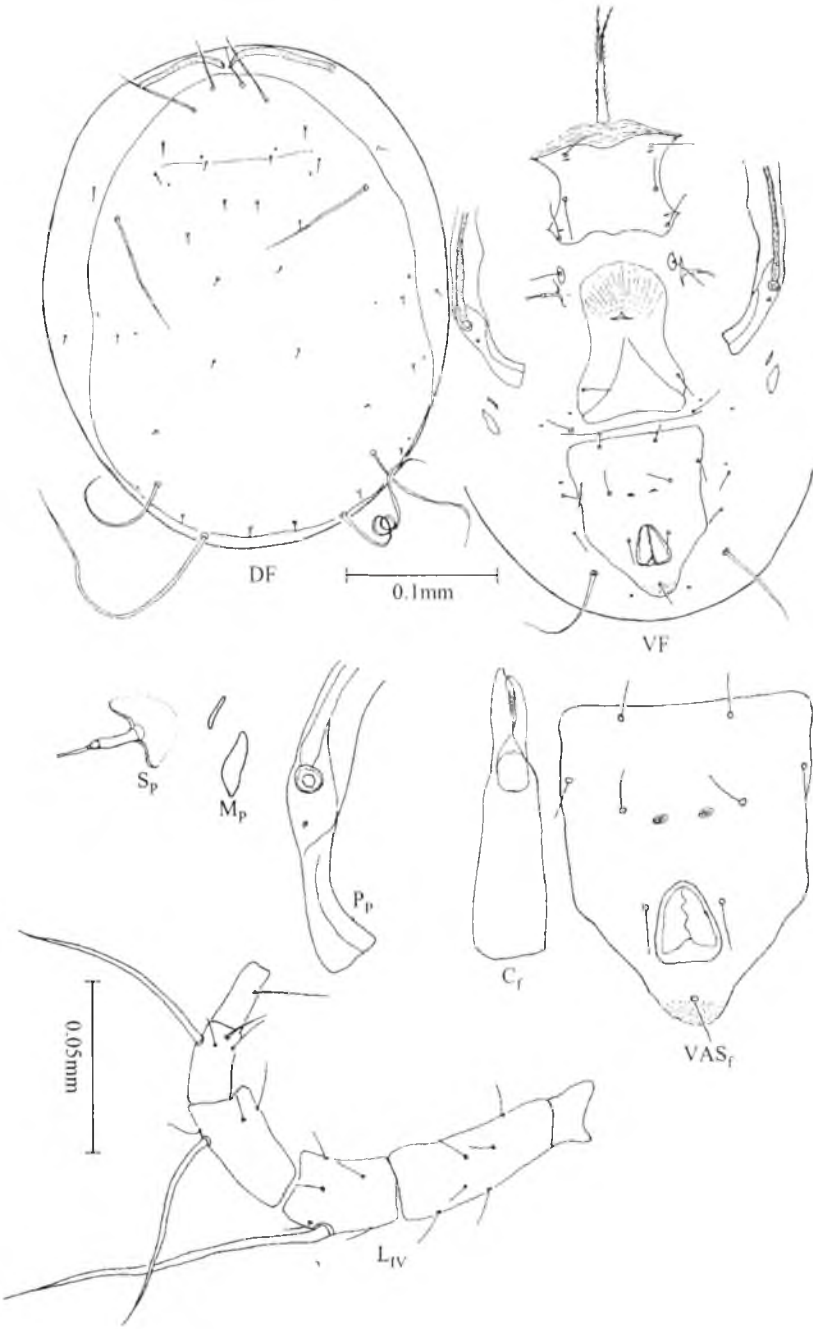


FIGURE 1. *Amblyseius (Amblyseius) apocynae* sp. nov.

genu III,  $2\frac{2}{2}\frac{1}{0}1$ ; tibia III,  $1\frac{2}{2}\frac{1}{1}1$ .

### Male

Unknown.

### Types

Holotype Female, India, Kerala, Kottayam, 16.IX.1992. ex-ornamental plant (Apocynaceae), Coll: C. Chinniah (89-6/1).

### Remarks

This new species resembles *Amblyseius muralidharani* Gupta (1986) in the shape of dorsal shield and chaetotaxy and short cervix of spermatheca, but differentiated from it by following characters 1. Smaller dorsal shield and shorter dorsal setae excepting 'V' and  $L_1$ ; 2. Sternal shield is also small, 3. Ventrianal shield without reticulations but reticulate in *A. muralidharani*. The structure of spermatheca also differs, the cervix is short with parallel walls and hence it is described as a new species.

### 2. *Amblyseius (Amblyseius) pseudorientalis* sp. nov. [Figs 2 and 2(a)]

#### Female

Dorsal shield smooth 324 long and 212 wide, with 4 pairs of prominent pores and 17 pairs of setae, of these V,  $L_1$ ,  $L_4$ ,  $M_3$  and  $L_8$  are long but not whip like. Setae  $M_3$  and  $L_8$  slightly serrate,  $L_1 > V$ ;  $L_3 > M_3$ ;  $L_8 > M_3$ ; other setae minute and smooth. Measurements of various dorsal setae V-22,  $L_1$ -38,  $L_4$ -62,  $M_3$ -66 and  $L_8$ -80. Setae  $L_2$ ,  $L_3$ ,  $L_5$ ,  $L_6$ ,  $L_7$ , 8-10 long each;  $D_1 = D_2 = M_1 = Clunal = S_2$  6-8 long each.  $D_3 = D_4 = S_1 = M_2$ -10 long each. Two pairs of sublateral setae on lateral integument, sternal shield long (70) than wide (62) with two pairs of visible pores and 3 pairs of sternal setae (20 long each). The 4th pair on integument, metasternal platelets not prominent. Genital shield 80 wide with a pair of setae (20 long). Space between genital and ventrianal shield 10 wide. Ventrianal shield 106 long and 80 wide with 3 pairs of preanal setae (16 long each) and a pair elliptical preanal pores. Four pairs of setae on the membrane around ventrianal shield, a thin folding also present in the space between genital shield and ventrianal shield. Length of setae V  $L_1$  40. Among metapodal platelets, only primary ones prominent (8 long) and the accessory pairs not visible.

#### *Gnathosoma* (cf)

Length of chelicera (100) normal and proportionate to body size, fixed digit of chelicera with 8-10 teeth and movable digit with 3-4 minute teeth. Peritreme extends anteriorly beyond vertical setae.

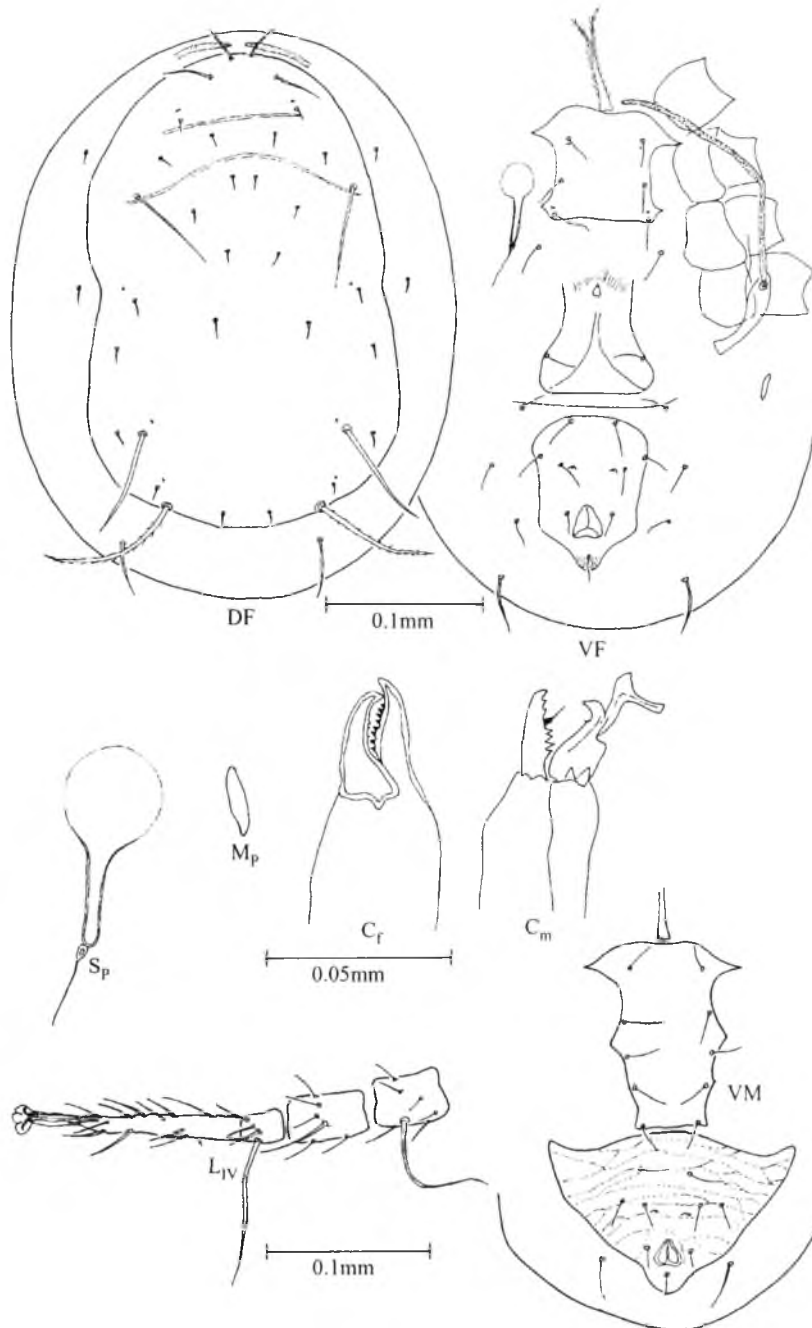


FIGURE 2. *Amblyseius (Amblyseius) pseudorientalis* sp. nov.

*Legs (LI-IV)*

Macrosetae present on genu I (40), genu II (32), genu III (42), tibia III (22), basitarsus II (24), genu IV (54), tibia IV (36) and seta on basitarsus IV (82) being the longest of all leg setae, The chaetotactic formula:

$$\begin{array}{ll} \text{genu II, } 2\frac{2}{0}\frac{1}{1}1; & \text{tibia II, } 1\frac{2}{1}\frac{1}{1}1; \\ \text{genu III, } 2\frac{2}{2}\frac{1}{1}0; & \text{tibia III, } 1\frac{1}{2}\frac{2}{1}0. \end{array}$$

*Male*

Dorsal Chaetotaxy as in female, ventrianal shield massive with reticulate patterns and three pairs of preanal setae and a pair of preanal pores. Spermatophoral process as figured.

*Types*

Holotype Female, India: Kerala, Pathananthitta, 13.X.1993; ex-undetermined forest shrub, Coll. C. Chinniah (No. 131/2-3) and one paratype slide, with 3 females, and one allotype male, collection data same as type.

*Diagnosis*

This new species resembles *Amblyseius orientalis* Ehara 1967 in general facies and structure of ventrianal shield and absence of metasternal platelets. However it is distinguished by the following characters.

1. Dorsal shield is much shorter than *A. orientalis*
2. Setae  $M_3$  and  $L_8$  are very shorter in new species measuring 66,480 respectively.
3. The structure of spermatheca clearly differentiates the new species from *A. orientalis*.

**3. *Amblyseius (Amblyseius) rubiae* sp. nov. [Figs 3 and 3(a)]***Female*

Dorsal shield smooth 321-330 long and 235-240 wide with 17 pairs of setae; setae 'V'  $L_1$ ,  $L_4$ ,  $M_3$  and  $L_8$  long ( $L_4$ ,  $M_3$  and  $L_8$  are whip like). Setal measurements being V-36,  $L_1$ -50;  $L_4$ -96;  $M_3$ -100;  $L_8$ -264-274;  $M_3 > L_4$ ; other setae very minute and the measurements are as follows,  $L_2 = L_3 = L_4$ -10 long each;  $L_6 = L_7$ -8 long each;  $D_1 = D_2$  = clunal setae-6 long each;  $D_3$ -4 long;  $D_4$ -10 long;  $D_4 > D_2 = D_1 > D_3$ ; median setae  $M_1 = M_2$ -6 long each. Two pairs of sublateral setae on the lateral integument,  $S_1$ , 10 long;  $S_2$  8 long. Dorsal shield has more than five pairs of distinct pores.

Sternal shield a little longer (80) than wide (70) with three pairs of sternal setae (34-36 long each) and a pair of lyriform pores just above the 3rd pair of sternal setae.

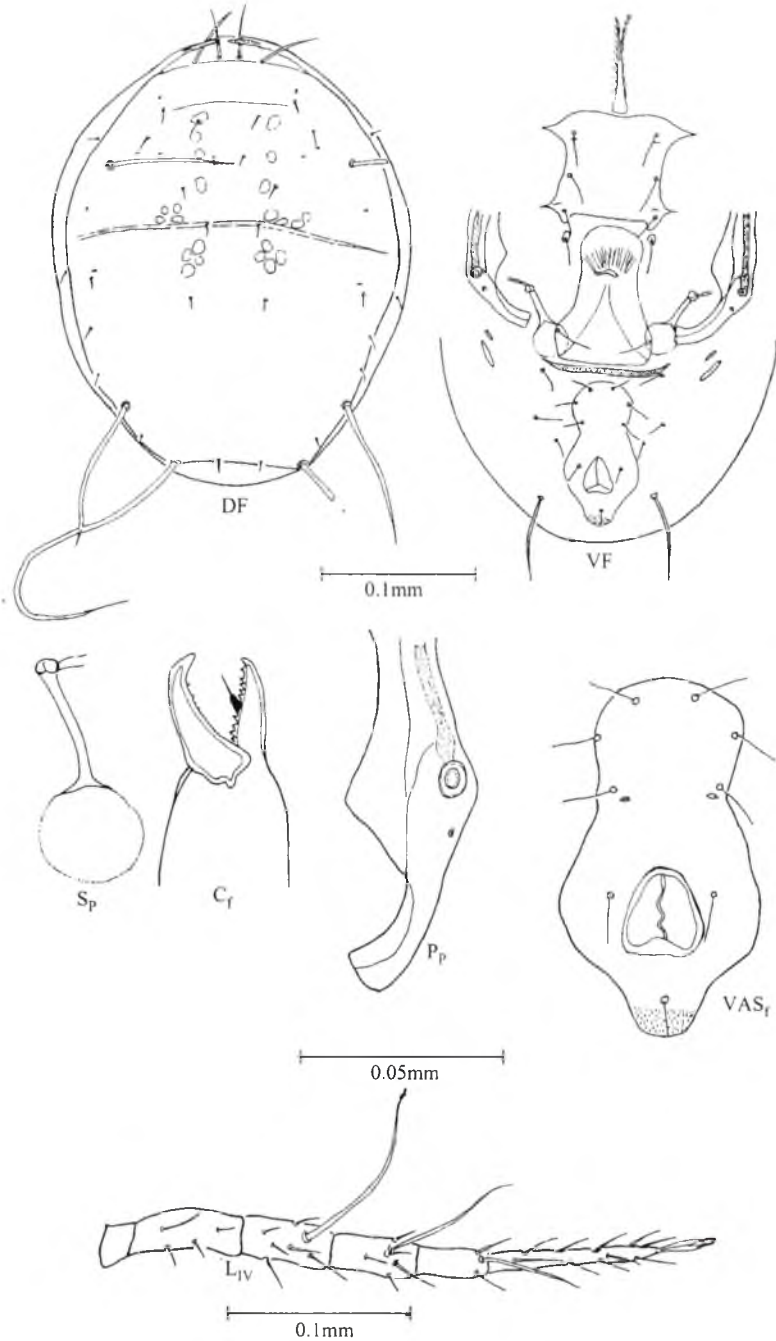


FIGURE 3. *Amblyseius (Amblyseius) rubiae* sp. nov.

Posterior margin of the sternal shield lobate and laterally hooked. Fourth pair of setae (26 long) present on a pair of distinct metasternal platelets. Genital shield 76 wide with a pair of setae 24 long. The ventrianal shield set apart by 8–10 from the genital shield and a prominent folding present in between. Ventrianal shield longer (104) than wide (60) with three pairs of preanal setae (14–16 long each) and a pair of prominent preanal pores just below 3rd pair of preanal setae. Ventrianal shield typically vase shaped. Four pairs of setae present around ventrianal shield on integument and length of  $VL_1$  being 28–30. Peritremal shield well sclerotized and the peritreme extending up to level of vertical setae, two pairs of metapodal platelets present. Primary platelets measuring 16–18 long and secondary one measuring 8–10 long. Spermatheca well developed with a strong and long cervix (28–30) and typically knobbed at base.

#### *Gnathosoma* (cf)

Chelicera normal and proportionate (104 long) to body size. The fixed chela with 8–10 strongly developed teeth and a prominent *Pilus dentilis* while movable digit bearing 2–4 teeth.

#### *Legs* (LI–IV)

Macrosetae present on genu of leg I (42) genu of leg II (36), Genu, tibia and basitarsus of leg III 40, 40 and 30 long respectively and on leg IV genu 114, tibia 88; and basitarsus 60 long. Macrosetae on genu IV being longest among leg setae. Leg chaetotactic formula:

$$\begin{array}{ll} \text{genu II, } 2\frac{2}{0}\frac{2}{0}1; & \text{tibia II, } 1\frac{2}{1}\frac{1}{1}1; \\ \text{genu III, } 2\frac{2}{1}\frac{1}{1}1; & \text{tibia III, } 1\frac{2}{2}\frac{1}{0}1. \end{array}$$

#### *Types*

Holotype female India: Kerala: Walayar forest, 4.X.1992, ex. Rubiaceae shrub., Coll: C. Chinniah; two paratype females, collection details same as type (Coll. No. 121/5).

#### *Remarks*

This new species closely resembles *Amblyseius coffeae* (Thamilselvi and Mohanasundaram, 1990) described from Munnar forest of Kerala region, in general facies and structure of spermatheca. However, it is clearly differentiated by the following characters.

1. The length of dorsal setae  $L_4$  is two times more longer (96) in new species than in *A. coffeae*.
2. The measurements of other dorsal setae also differs between these two species.
3. A strong fold is present in between genital and anal shield in new species which, is absent in *A. coffeae*. Hence it is erected as new species.

#### 4. *Amblyseius (Paraphytoseius) apocynaevagrans* sp. nov. [Figs 4 and 4(a)]

##### *Female*

Dorsal shield 260 long, 114 wide, elongate, incised at level of  $L_4$  with 13 pairs of setae. Setae  $V$ ,  $L_1$ ,  $L_4$ ,  $L_8$  and  $M_3$  long, thick and serrate, measuring 32–34, 70–80, 100–106, 100–104 respectively, while other setae measuring  $D_1 = D_2$  4–6,  $D_3$  8–10,  $Cl_4$ ;  $M_1$ ,  $M_2$  4–6;  $L_2 = L_3$  18;  $S_1$  and  $S_2$  serrate measuring 40–50, 35–40 respectively. A pair of large round solenostomes present just near  $M_1$  on the proscutum. Sternal shield 80 long, 72 wide with 3 pairs of sternal setae (36), 4th pair (30) lie on metasternal plates. Genital shield 76 wide, with a pair of setae (34). Genital shield and ventrianal shield 34 apart with a thin integumental folding in the interspace. Ventrianal shield 90 long, 46 wide with 3 pairs of preanal setae (20); and 4 pairs of setae present on membrane around the ventrianal shield,  $V L_1$  70 long, thick and serrate. A pair of very thin and long (34) metapodal platelets present.

##### *Gnathosoma (cf)*

Fixed digit of chelicera with more than 10 strong teeth and a prominent *pilus dentilis* movable digit bidentate.

##### *Legs (II–IV)*

Macro setae present on Leg (IV) Genu (24) tibia (40), basitarsus (46) and distitarsus (36) all with knobbed tip—The chaetotactic formula as follows

$$\begin{array}{ll} \text{genu II, } 2\frac{2}{1}\frac{1}{0}1; & \text{tibia II, } 1\frac{2}{1}\frac{0}{2}1; \\ \text{genu III, } 1\frac{2}{2}\frac{1}{0}1; & \text{tibia III, } 1\frac{2}{1}\frac{1}{1}1. \end{array}$$

##### *Male*

Dorsal chaetotaxy similar to that of female. Spermatophoral process as figured. The setae on leg IV as follows, genu 18; tibia 24; basitarsus 28; and distitarsus 28.

##### *Types*

Holotype female, marked on slide along with two more females, India: Kerala: Kottayam Dt., 16.IX.1992. ex. Undet. Ornamental Plant (Apocynaceae), Coll. T. K. John (No. 89/4). Several paratype females, and an allotype male, collection data same as type (No. 89/1, 2, 3; 88/1, 2).

##### *Remarks*

This species resembles *Amblyseius (Paraphytoseius) multidentatus* Swirski and Schechter (1961), in dorsal chaetotaxy and shape of dorsal shield, presence of capitale macrosetae on genu, tibia, basitarsus and distitarsus on leg IV. However, it is clearly

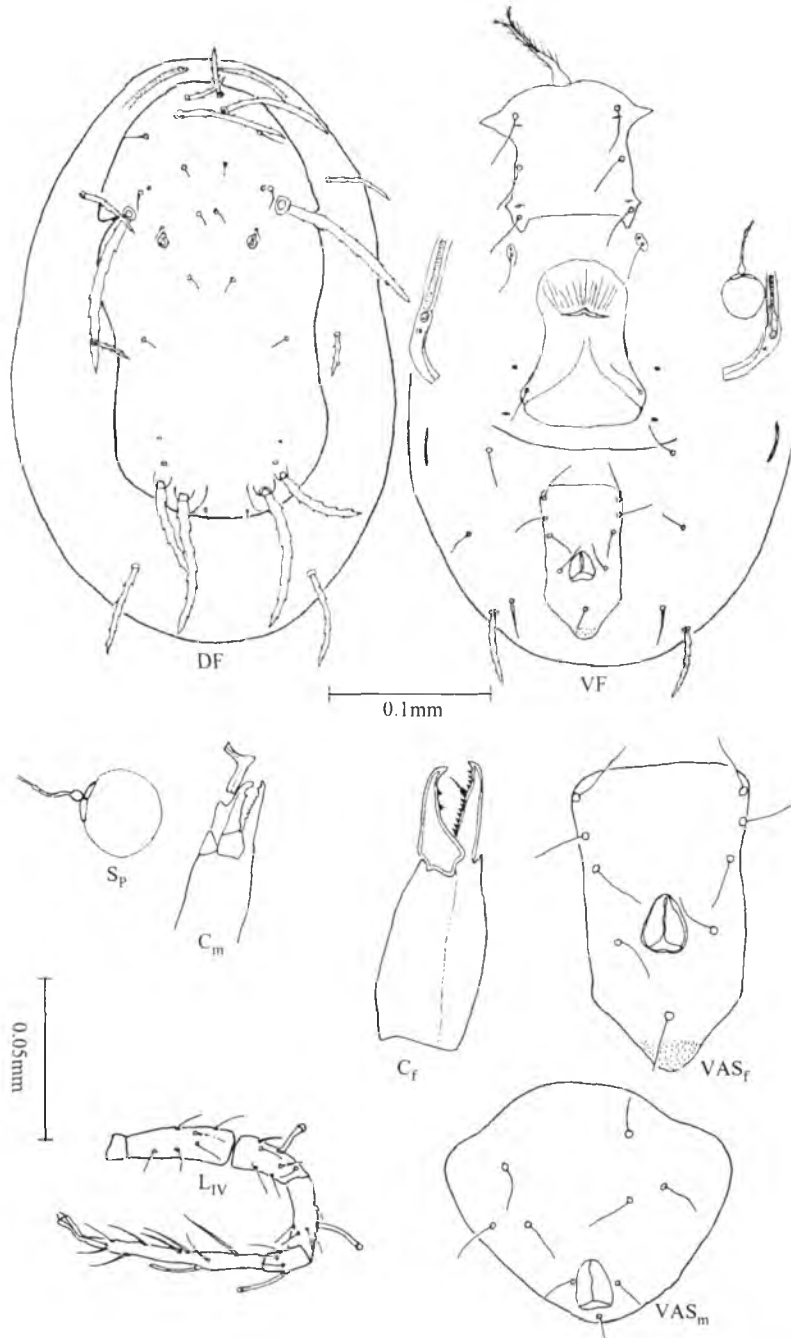


FIGURE 4. *Amblyseius (Paraphytoseius) apocynaevagrans* sp. nov.

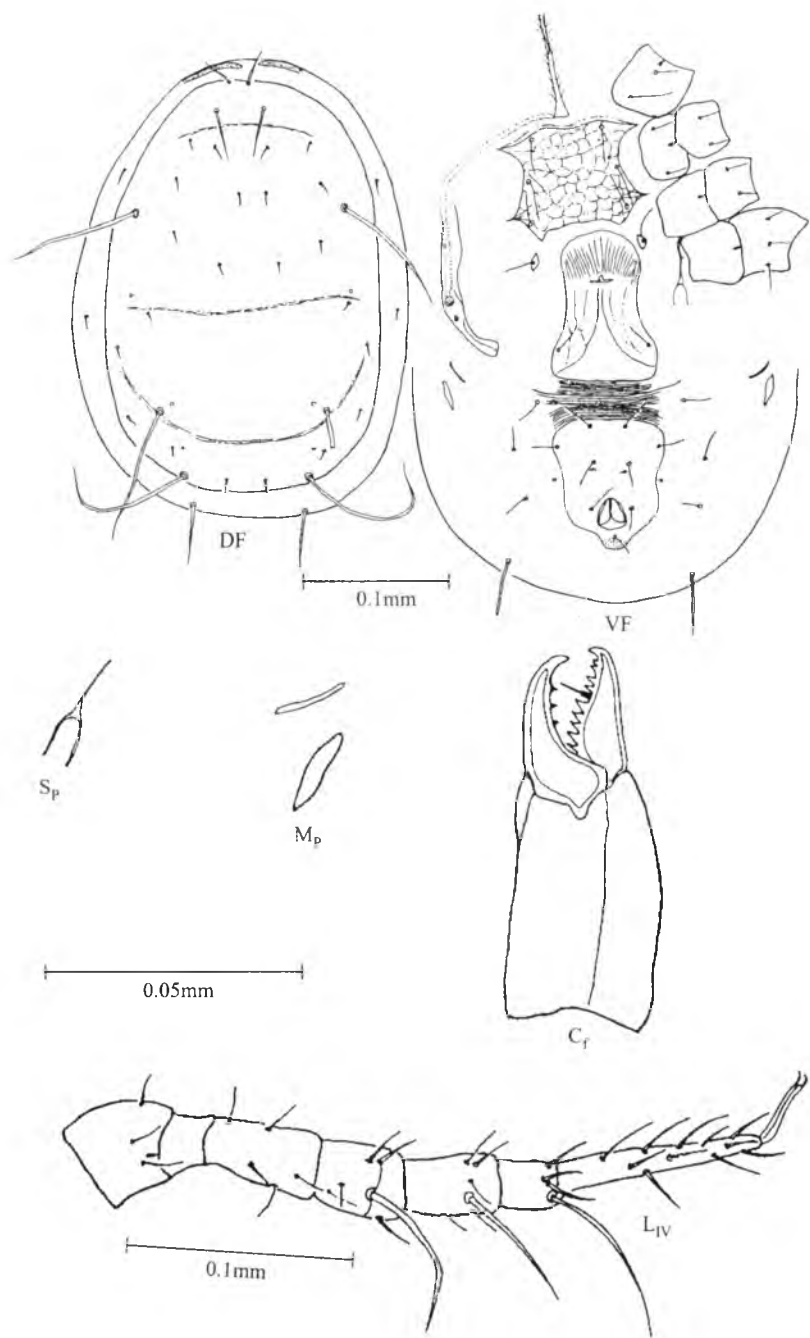


FIGURE 5. *Amblyseius (Proprioseiopsis) keralaensis* sp. nov.

differentiated by the following characters. (1) Dorsal shield is much smaller and almost all dorsal setae is shorter in length in new species. (2) The dorsal setae  $L_7$  absent in the new species on proscutum. (3) Moreover, dorsal shield is not rugose. (4) The post lateral angulation of sternal shield is very prominent, shape of the genital shield is quite different. i.e. the posterior part is truncate in new species. The metapodal platelet is very thin and long. (5) The structure of spermatheca is quite different in the new species. The structure of spermatophoral process of male is also different in the new species.

**5. *Amblyseius (Proprioseiopsis) keralaensis* sp. nov. [Figs 5 and 5(a)]**

*Female*

Dorsal shield smooth, 277 long and 188 wide, well sclerotized with 16 pairs of setae,  $V$ ,  $L_1$ ,  $L_4$ ,  $M_3$ ,  $L_8$  long, other setae minute. Only 3 pairs of dorsocentral setae present (excluding verticals and clunels),  $D_4$  absent, 3 pairs of median setae 8 pairs of lateral setae and 2 pairs of sublateral setae on lateral integument which is also sclerotized. Measurements of various dorsal setae  $V$  18,  $L_1$  28,  $L_2 = L_3$  4–6 long each,  $L_4$  90,  $L_4 = L_6 = L_7$  6–8 long;  $L_8$  100 long  $D_1 = D_2 = D_3 =$  clunals 4–6 long;  $M_1 = M_2$  6–8 long,  $M_3$  94 long;  $S_1$  10–12 long;  $S_2$  8 long, sternal shield wider (80) than long (68) with 3 pairs of sternal setae (26 long each), 4th pair of sternal setae (18 long) on a pair of prominent and well sclerotized metasternal platelets. Sternal shield reticulate with concave lateral and posterior margins. Genital shield 70 wide with light reticulate patterns and a pair of genital setae (14 long). Genital and ventrianal shield 30 apart and a very thin folding is present in the interspace. Ventrianal shield pentagonal, smooth, 88 long and 78 wide with 3 pairs of preanal setae (16 long) and a pair of preanal pores, 4 pairs of setae on membrane surrounding ventrianal shield, setae  $V L_1$  48 long. Two pairs of metapodal platelets, primary one 16 long, the secondary one also equally long (16) despite very thin. Structure of spermatheca as illustrated. Length of chelicera (100) normal and proportionate to body size. Fixed digit of chelicera with 10 teeth which are very strong and prominent, while movable digit with three fine teeth. On legs the macrosetae present on genu I, genu II, genu III, genu IV, tibia IV and basitarsus IV, each measuring 36, 32, 32, 66, 44, 76 long respectively.

*Males*

Not known.

*Type*

A holotype female marked on slide along with 2 larvae, India: Kerala: Kottayam Dt., 17.X.1992, ex. Undetermined ornamental shrub Coll. C. Chinniah (No. 137/2).

*Remarks*

This new species resembles *Amblyseius (Proprioseiopsis) arunachalensis* Gupta (1986) in dorsal chaetotaxy, sternal shield being wider than its length and with

reticulate patterns. However, it is distinguished by the following taxonomic characters measurements of body size and length of setae is very shorter ( $L_4$ ,  $M_3$ ,  $L_8$ ) one third long in new species structure of spermatheca also differs in these two species. The primary and secondary metapodal platelets are equal in length in new species but the length of these two plates varies in *A. propriozeiopsis*.

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## Propagation Methods of *Ceutorhynchus portulacae*, a Potential Biocontrol Agent of *Portulaca oleracea* L. Procedures and Cost

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**ABSTRACT:** The curculionid weevil *Ceutorhynchus portulacae* Marshall was identified as a potential natural enemy that could be utilised for biological control of the crop weed *Portulaca oleracea*. Augmentative releases of bioagents for timely suppression of the target weed, without any crop loss is an important factor in any bio-control programmes of crop-weed. This necessitates development of an efficient mass production technique, whereby availability of adequate bioagents is ensured for timely field releases. The present paper describes the various mass rearing methods standardised for *C. portulacae* and discusses the most effective and economical mass rearing method that could be utilised for propagation and effective suppression of *P. oleracea*.

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**KEYWORDS:** *Portulaca oleracea*, *Ceutorhynchus portulacae*, mass multiplication.

### INTRODUCTION

*Portulaca oleracea* L, a plant of South American origin, ranking as the ninth worlds weed is reported to infest about 85 crops in 45 countries of the World (Holm *et al.*, 1977). In tropical countries, it is of considerable importance in many upland crops including vegetables, maize, cotton, groundnut, sorghum, sugarcane, sunflower and rice (Waterhouse, 1993). In India, it ranks as a serious problematic weed of vegetables, vineyards and banana orchards, especially during the rainy seasons (Chadha *et al.*, 1995; Mandal, 1990). The weed also acts as alternate host to varied pests and diseases (Holm *et al.*, 1977) and has allelopathic effects to many crops (Leela, 1992). The curculionid weevil *Ceutorhynchus portulacae* Marshall was identified as a potential indigenous biocontrol agent that could be utilised for the biological suppression of the weed (Ganga Visalakshy and Jayanth, 1997). The biology, host specificity

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and potential impact indicated that the insects could suppress the weed under field conditions. The larvae mines through the leaves causing wilting and drying of the plant. The adults are also voracious foliage feeders. Under severe infestation the combined feeding of the adult and larvae causes complete defoliation of the plant.

Studies have revealed that for vegetables, the critical weed free period (the maximum duration that weeds can be tolerated without affecting crop yield) is determined between 4–8 weeks after transplanting (Zimdahi, 1980; Mani *et al.*, 1968). This indicates that effective biological suppression of purslane weed has to be attained within this period, which necessitates an early high population build up of the weevils. Augmentation of the bioagents is one of the methods recommended for high population build up and timely suppression of many weeds. Attempts to augment phytophagous insects for control of weeds have been rare. Known examples of augmentative efforts are the sequential releases of the weevil *Rhinocyllus conicus* Froelich, use of field nurseries for the propagation of *Trichosirocalus horridus* (Panzer), mass rearing of the moth *Bactra venosana* Zeller, *Oidaematophorus monodactylus* (L) and *Agapeta Zoe-gana* L for the control of *Cardus nutans* L, *C. thoeneri* (Weimann), *Cyperus rotundus* L, *Convolvulus sepium* L and *Centaurea maculosa* Lamarck respectively (Surles and Kok, 1976; Stoyer and Kok, 1986; Frick and Chandler, 1978; Parella and Kok, 1979). This paper reports the different rearing procedures that could be utilised for mass multiplication of *C. portulacae*, whereby effective suppression could be achieved.

## MATERIALS AND METHODS

### Mass multiplication in plastic jars

*C. portulacae* was multiplied under laboratory conditions in clean, transparent plastic jars (11 × 14 cms) which were modified as insect rearing cages. The central part of the lid (5 cms diameter) was cut off with a heated knife and a brass wire-mesh was fixed by heat-sealing, to provide ventilation to the jars. A large number of such jars were made and used as cages for oviposition, larval rearing and pupation, depending on the need.

A week old *C. portulacae* adults were released at the rate of 5 and 10 pairs per jar to which *P. oleracea* bouquets were kept. The bouquets were made from one-month-old *P. oleracea* plants grown under glass house conditions. Twigs of 10–15 cms length were cut and brought to the laboratory. The base of the twigs was wrapped with thick cotton, held together by rubber band. The cotton swab was moistened as and when needed to prevent the exposed twigs from wilting. The bouquets exposed for oviposition were replaced once in 3 days. The process was repeated till 12th week after emergence of adults, after which the adults were discarded and the process was continued with newly emerged adults.

The bouquets exposed to adults of *C. portulacae* were kept in a separate, clean, aerated plastic jar, for hatching. The larvae were provided with fresh, unexposed bouquets for feeding, as and when needed. As it was difficult to transfer the larvae from one bouquet to another, the following procedure was adopted. The bouquets from which larvae are to be transferred was cut at the base from the moist cotton swab

and kept at the bottom of another clean dry jar. Fresh bouquets were introduced into this jar. As the leaves dried up, the larvae migrated to the fresh ones. The old bouquets were removed after a day. This process was continued till the larvae completed their development.

The full-grown larva falls down to the base of the jar, normally aggregating near the cotton swab. These were collected daily and released into pupation cage, in which a few twigs of purslane were also provided. The jar was filled up to 5 cms from the base with sterilised soil and covered over with dried purslane leaves. The soil was moistened up 1 cm from the top before releasing of the matured larvae. Once all the released larvae have entered the soil and pupated the jars were kept for adult emergence. As soon as adults started emerging, the soil was slightly moistened, to enhance adult emergence, which were collected daily.

#### **Mass multiplication in plastic cages**

In addition to the above method, the insects were multiplied directly on purslane plants, grown in pots. For this, purslane plants were grown by sowing seeds on seed pans (50 cm) in glass house. Pots with month old plants were brought to the laboratory and enclosed in plastic cages. The cages were made using acrylic plastic sheets. The plastic sheets were rolled into cylinders of 40–45 cm diameter and 30 cm ht. The cut ends were pasted with an adhesive. Wooden frames that can be tightened by screws supported the top and bottom ends of the cylinder. The bottom end of the cage was kept open over the pots, covering the plants while the top part was covered over by a nylon mesh held tight by the wooden frame. A slit of 5 cm diameter was made in the nylon mesh, through which the adults were released. The slit was covered by a thick sponge, to prevent escape of adults. Adults at the rate of 5, 10 and 15 pairs were released into each cage. The bottom part of the cage was kept firm by adhering soil to the sides of the cage. After 10–12 days, the cages were removed and the leaves with larvae were collected and released to the pupation cages, as mentioned above for adult emergence. The pots once opened for collection of leaves were discarded.

#### **Mass multiplication by using large wooden cages**

Instead of using the plastic cages, mass multiplication of *C. portulacae* was attempted by enclosing purslane plants in large wooden cages. The cages were of 40×40×60 cms ( $l \times w \times ht$ ), with nylon wire mesh on three sides and top and a sliding glass front. Seed pans with purslane plants of a month old were kept inside the cage (more egg laying was obtained when a month old weed were exposed). To these cages, a week old adults were released, at the rate of 5, 10 and 15 pairs per cage. By one week, mines start appearing on the leaves. These were collected once in three days and released into pupation cages, where a twig of purslane was also provided. The plants were watered once in 3–4 days. This was continued till all the mined leaves were collected. The cages were frequently kept outside, exposed to the field conditions.

TABLE 1. Details on the different methods of mass multiplication of *C. portulacae*

Sl. No.	Treatments	No. of adults collected/jar/ cage/week	Recovery of the released adults (%)	Man hours needed (per day/jar or cage)	Approximate Cost (in Rs)		Total cost (in Rs)
				(Technical)	Labour cost (in Rs)	Material cost	
1.	Jars	75-100	50-60	60-95 mts	Labourer 50 Technician 120 Total 170	Pet jars (6) Brass mesh Pots (6) Total	420 50 120 590
2.	Acrylic cage	125-150	30-40	30-45 mts	Labourer 28 Technician 40 Total 68	Pots (2) Cage material Wooden frame Pet jar Adhesive, Cloth etc Total	40 110 35 70 25 280
3.	Wooden cage	300-500	80-90	5-10 mts	Labourer 28 Technician 10 Total 38	Pots (2) Wooden cage Pet jar Total	40 1300 70 1410
							1448

## RESULTS AND DISCUSSION

The details of the different methods of rearing in relation to the cost involved in making a unit (oviposition, larval rearing and pupation units), labour involved (time required for maintenance of plants and culture) and the quantity of bioagents produced are given in Table 1.

Rearing in plastic jars was found to be labour intensive and time consuming (Table 1). *P. oleracea* being a succulent plant requires daily attention in maintenance, by this method. Inability in attending the culture daily causes decay of the bouquets. This resulted in high mortality of the eggs and drowning of the developing larvae and adults, thereby significantly reducing the culture material at many times. The adults are active insects that make short jumps even on slightest disturbance. Losses of adults while changing the bouquets were also encountered by this method. Releasing of 5 pairs was found to be the optimum dosage as higher dosages resulted in reduced larval collection.

Multiplication in plastic cages was found to be better than rearing in plastic jars. Compared to the above method this technique yielded more adults and also time saving as it required only collection of leaves with larvae, once in 10–12 days (Table 1). Releasing of 5 pairs of adults was found to be optimum number, as higher dosage led to more foliage feeding and reduction in larval mines. This method, however resulted in wastage of the plant material as all the leaves of the exposed plants may not harbour larvae during the time of collection. Also, most of the released adults escape when the cage was removed, resulting in loss of viable adults. Though the cost of making a unit was found to be cheaper compared to other units, it was a continuous recurring expenditure as the frame and the acrylic material could be used for two or three times only.

Rearing of adults in open cages was found to be the most suitable method. As mentioned above, releasing 5 pairs of adults per cage yielded more larvae, as higher dosage led to more foliage by adults. By this method about 200–300 mines could be collected once in three days, which required only 5–10 minutes (Table 1). Unlike the acrylic cage method, this method helped to utilise all the leaves of the exposed plant. Hence, there was no loss of plant material. A total of 800–1000 adults could be reared from a single one month old purslane plant. Unlike observed in the acrylic cage, leaves could be collected by moving the front glass pane which prevented loss of released adults jar. The adults were found moving on the sides of the cages after all the leaves were removed. About 80–90% of the released adults could be collected back easily and reused. Though the cost involved in making the unit was comparatively high than to other units it is a permanent structure which needs no recurring expenditure. Also taking into account the number of adults produced per unit the material cost works at par to the other units.

Continuous mass multiplication of bioagents under controlled ambient environmental conditions has been reported to affect the fertility, fecundity, longevity and searching behaviour, resulting in gradual deterioration and reduction in their effectiveness under field conditions. For, rearing of *Zygogramma bicolorata* Pallister and *Paracheates*

*pseudoinculata*, bioagents of parthenium and Eupatorium weed continuously under laboratory conditions was reported to cause significant reduction in the fecundity, hatching, survival and sex ratio of the adults produced (Jayanth and Geetha Bali, 1996; Ganga Visalakshy and Jayanth, 1998). Our studies revealed that continuous rearing of *C. portulacae* in wooden cages exposed to outside environmental conditions was found to cause no deterioration in the functional potential of the insects (Ganga Visalakshy, 2000). Exposure of the insects to the fluctuating environmental conditions could have been one of the factors that helped to maintain the quality of the bioagents produced. The objective of the present study was to produce large number of good quality *C. portulacae* adults and developmental stages in an economical way, within a short time for timely field releases, to hasten the population build up and suppression of the weed, *P. oleracea*. Of the methods described, multiplication in large wooden cages was found to be the best suitable method as it satisfies all the above requirements.

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## Effect of Caffeine on Wing Morphogenesis in *Drosophila melanogaster*

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**ABSTRACT:** Imposition of stress during critical period(s) of development is known to cause morphological defects in various organisms including *Drosophila*. We now report here the production of abnormal wing phenotypes in *Drosophila* induced by larval feeding of caffeine. Some of these were phenocopies of known mutants such as *clubwing*, *curved* and *outstretched*. Variants of the above phenocopies were also observed. One or both wings were affected. However, no sex-wise preference was observed for the expression of abnormalities.

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**KEYWORDS:** caffeine, *Drosophila*, phenocopy.

Genomic stressors of a wide range are known to produce morphological defects in *Drosophila* as well as in other organisms (Goldschmidt and Peternick, 1957). Some of these defective organisms are referred to as phenocopies because morphologically they tend to resemble specific mutant phenotypes, but without any stable genetic change. A number of physical and chemical agents have been known to induce phenocopies in *Drosophila* (Gloor, 1947; Rapport, 1973). One of the most studied of these agents is heat shock. It has been suggested that the phenocopying effect of heatshock is the result of its action at the level of transcription of particular genes or at the level of their mRNA or both (Mitchell and Petersen, 1981). Also there could be some effect on protein turnover (Petersen and Young, 1989). In short, a phenocopy originates from a lack of recovery of specific functions in time to participate in continuing development. In other words, a transitory repression of a particular activity at any one of the above levels is equivalent to a lack of function or an abnormal function in a typical mutant, and the phenotype will depend on the particular gene whose activity was repressed and thus related to specific developmental stage.

Earlier studies show that caffeine (1,3,7 trimethylxanthine) is capable of inducing puffing at some of the major heat shock loci in the polytene chromosomes of

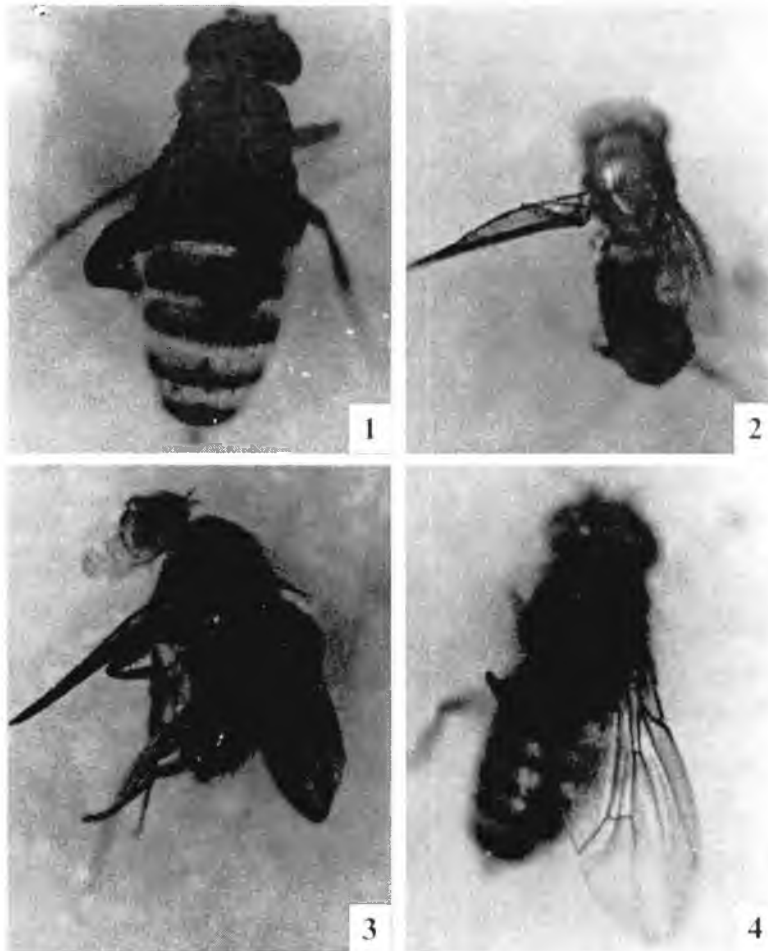
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*D. melanogaster* (Srivastva and Bangia, 1985). In view of this as well as because of its known inhibitory effects on particular developmental stage in this organism (Mohamed and Nair, 1991), the phenocopying potential of this methylxanthine was investigated.

Oregon K. Strain of *D. melanogaster* used in the present study was obtained from the Drosophila Stock Centre, University of Mysore. The flies were grown on a standard culture medium containing wheat flour (substitute for corn meal), molasses, agar and yeast with Nepagin as a fungicide. Age-synchronised larvae were used for all the experiments and caffeine (1000 µg/ml) was administered through larval food. The parental (obtained from caffeine-fed larvae) and  $F_1$  (progeny got by inbreeding the above parental flies) generations of flies were analysed for abnormal phenotypes. For inbreeding, phenotypically normal-looking parental flies were used. A few crosses involving abnormal phenotypes with normal-looking flies were also made.

Administration of caffeine during any of the larval instars induced phenocopies. However, percentage-wise, production was higher when the larvae were treated during the first or second instars (2–3%), rather than during the third instar (–0.5%). Some typical phenocopies produced were those of *clubwing*, *curved* and *outstretched* or their variants (Figs 1 and 2). A variant of *narrow* was also observed (Fig. 3). A few abnormal phenotypes could not be classified into any specific categories (Fig. 4). Although in typical phenocopies, both wings were affected, in many of the variants, only one wing was of the abnormal type. No sex-wise difference was observed in the expression of the abnormalities. All the defective flies were flightless, even where the wings themselves were mobile. The percentages given above are with respect to the parental category of flies. Although some abnormal wing phenotypes arose in the  $F_1$  category as well, this aspect needs further analysis. However, it could be stated that there was no definite pattern (with respect to category or frequency) at which the different phenocopies arose in the progeny of inbred parental flies or even in the progeny of the small number of crosses involving abnormal phenotypes with normal flies of the opposite sex, so as to suggest any strict genetic basis for their appearance.

The wing imaginal discs in *Drosophila* are set aside during embryonic development as a discrete anlage of 20–30 cells each (Bate and Arias, 1991). These grow throughout larval life, forming by late third instar, sacs of approximately 52 000 epithelial cells. During metamorphosis, the imaginal discs, including the wing disc, undergo a complicated series of morphological changes and differentiate to form the precise pattern of the respective adult tissue types. This differentiation, to a large extent, is induced by the large hormone pulse of 20–HE which occurs at the end of the third larval instar. co-ordinate expression of many genes, such as *engrailed*, *hedgehog*, *decapentaplegic*, *wingless*, *fringe* and *scalloped* is crucial for the normal development of wings. These gene products are mostly controlling and signalling molecules and are important in cell interactions as well as in patterning; while some like *decapentaplegic* act as a gradient morphogen, others act to regulate transcription and control the production of the next protein in the sequence (Zecca *et al.*, 1995). Interference at



FIGURES 1-4: (1) Phenocopy of *clubwing*; (2) Variant of *outstretched*; (3) Variant of *narrow*; (4) An abnormal phenotype with left wing very short and highly wrinkled.

the level of production or function of any of these molecules could lead to abnormal morphogenesis.

Although caffeine is known to have multifarious effects on cells, from the point of view of wing morphogenesis, the following findings made on the action of this methylxanthine could serve as a guideline for future studies:

- (i) inhibition of DNA, RNA and protein syntheses, apparently by binding onto single stranded regions of nucleic acids (Bilinski *et al.*, 1973).
- (ii) increasing the level of cAMP, either because of the rise in intracellular  $\text{Ca}^{2+}$  levels and the resultant increase in adenylcyclase or because of the inhibition of 3-5 cyclic nucleotide phosphodiesterase (Cohen *et al.*, 1991).

- (iii) affecting phosphorylation levels of proteins: e.g. caffeine has been shown to abolish the phosphorylation of Chk1 and attenuate this pathway of checkpoint control of G2/M transition. This action of caffeine could very well be because of its known ability to stimulate phosphatase, either directly or indirectly.
- (iv) reducing ecdysteroid titres in *Drosophila* larval haemolymph (Mohamed and Nair, unpublished data).

Perhaps of relevance in this context is also the finding (ultrastructural studies, to be reported elsewhere) of mitochondria which suffered large scale degeneration in the indirect flight muscles of the *outstretched* phenocopy. It is known that mitochondria are important in calcium sequestration in muscle fibres and this is all the more so in the case of indirect flight muscles of this organism, which have very little of sarcoplasmic reticulum, another important calcium sequestering organelle.

#### ACKNOWLEDGEMENTS

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## Environmental Interactions of Pesticides: Synergism of Methomyl by Simazine Against the House Fly, *Musca Domestica* L.

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**ABSTRACT:** The potential effect of methomyl and its interaction with simazine (a herbicide) on CTB strain of the house fly, *Musca domestica* was evaluated. 3-day old adults were exposed to the methomyl and its mixture with simazine and the acute toxicity was determined at 24 h. The synergistic effect of PBO was also investigated and compared with simazine. Unsynergised methomyl gave topical acute LD<sub>50</sub> values of 88.65 ng/fly after 24 h. When applied in a mixture simazine synergised methomyl at ratio 5:1 and greater. PBO also showed a similar pattern of synergism. The cotoxicity coefficients were estimated and isoboles were plotted to analyze and compare the interaction in both cases. © 2001 Association for Advancement of Entomology

**KEYWORDS:** *Musca domestica*, methomyl, Toxicity, simazine, synergism.

The house fly, *Musca domestica* L. is a synaptic pest of human and domestic animals (West, 1951; Service, 1986). It transmit a large number of diseases to man owing to their habits of visiting, almost indiscriminately, faces and other unhygienic matter and human food (Saleem *et al.*, 1993). Pathogens are spread by the flies contaminated feet, body hairs and mouthparts. In addition, they vomit during feeding and frequently defecate on food. Over 100 different pathogens have been recorded from house flies, atleast 65 of which are known to be transmitted. It can also transmit viruses such as poliomyelitis, trachoma etc. and infectious hepatitis as well as rickettsiae such as Q fever (*Coxiella burnetii*) and numerous bacterial diseases.

The house fly was reported to have developed resistance to different categories of insecticides in several countries (Price and Chapman, 1987; Keiding and Jespersen, 1991). Therefore, in order to have the same level of control, the amount of the insecticide used needs to be increased. This will inevitably lead to both increase in cost and also to the possibility of some insecticide accumulating in the environment. Continuous use of certain insecticides does have some disadvantages, as new insecticide and or combination of biologically active compounds need to be tested.

TABLE 1. LD<sub>50</sub>, Confidence limits and Regression equation

Chemical/synergist	LD <sub>50</sub> (ng/fly)	Confidence limits		Regression equation	$\chi^2$ (df)
		lower	upper		
Methomyl	88.65	81.10	96.91	$Y = -3.86 + 4.50X$	6.92(3)
Simazine	5724.62	4316.49	7592.11	$Y = -5.78 + 2.86X$	10.89(3)
PBO	345.79	295.71	404.36	$Y = -1.63 + 2.61X$	5.79(3)

The study of insecticide/herbicide synergism is also warranted from environmental perspective. Synergists have been used to study and combat pesticide resistance for ca. 45 years. The synergists were initially developed for use with pyrethrin, but have since been observed to synergise some other compounds including methomyl. There has been mounting interest in the use of synergists to reduce some resistance incidents by combat applications (Brown and Brogdon, 1987). The use of PBO, sesamex, simazine and other compounds to suppress resistance to different compounds has been documented in various out lets (Sun and Johnson, 1965; Wilkins and Khalequzzaman, 1993). Thus the objectives of this study were to assess the interaction between simazine and methomyl on the house fly, *M. domestica*.

The CTB an insecticide susceptible strain of the house fly was collected from Roussel Uclaf, U.K. The stock cultures were maintained at 25 °C in a mesh cage (50 × 30 × 30 cm). The adults were provided with 5% milk-sugar solution as food. Adult females laid eggs in a food cup (250 ml) having larval food (yeast, milk powder, bran at ratio 1 : 2 : 10 mixed in 500 ml of distilled water). When the flies emerged, they transferred to a separate cage each day, so that known age groups could be used for bioassay.

Required quantity of each insecticides were weighed on an electric balance using little aluminium foil boat and placed in a clean, well dried volumetric flask (10–25 ml). The required amount of solvent (acetone) was added to the flask to dissolve the insecticide and shook well for uniform mixing of the solution. In case of simazine it dissolved in a few drop of dimethylsulphoxide (DMSO) and then added required quantity of acetone. Five doses of methomyl (50, 80, 100, 120 and 150 ng/fly) were applied topically on 3-day old house flies. A mixture prepared in mass ratio of methomyl-simazine and methomyl-PBO were also applied. LD<sub>50</sub> for each ratio was calculated to compare the synergism.

The bioassay were determined by micro-topical application technique using a programmable Arnold Micro-Applicator (Barkard) and a micro syringe (1 ml) with canulae (G-36 × 3) using a procedure of Georghiou and Bowen (1966). A micro syringe calculation using mercury (Hg) was done before the tests. The 3-day old adults were removed from the rearing cages with an aspirator filled on vacuum suction device and collected in glass tube (2.5 cm). Treatment was made by applying 1.0 µl droplet of solution of an insecticide to the dorso-thoracic region of insect under CO<sub>2</sub> anesthesia. Each experiment was accompanied by a control. Each individual treatment consisted about 50 insects with 5 replications. After treatment the flies were placed into plastic

TABLE 2. LD<sub>50</sub> and cotoxicity coefficient of mixtures of methomyl (M) and simazine (S) or piperonyl butoxide (PBO) at different ratios applied topically to the house fly

Chemicals	Ratio	LD <sub>50</sub> (ng/fly)				CC
		M + S or PBO	M	S or PBO		
M:S	1 : 5	490.40	92.05	408.70		108
	1 : 10	465.97	69.63	696.35		127
	1 : 20	1126.27	53.63	1072.63		165
	1 : 40	1974.67	38.71	1935.95		229
M:PBO	1 : 5	749.26	68.10	681.10		130
	1 : 10	945.01	59.06	885.94		150
	1 : 20	1041.94	49.61	992.32		179
	1 : 40	1576.34	50.83	1525.50		174

Cotoxicity coefficient.

cups which contained cotton wool soaked in milk + sugar solution and placed in an incubator at temperature 25 °C.

Initial bioassays were carried out to established the effect of single treatments. The topical LD<sub>50</sub> of adult house fly was 88.65 ng/fly for methomyl treatment whereas, it was 5724.62 ng/fly for simazine treatment (Table 1).

The mixtures of methomyl and simazine were used in different ratios keeping methomyl constant at 88.65 ng/fly. The results (Table 2) indicate that simazine has some synergistic effect with methomyl to the house fly. The cotoxicity coefficients shows that when applied in a mixture simazine synergised methomyl at ratio 5 : 1 and greater. Below this the LD<sub>50</sub> for methomyl with simazine did not show any response. The LD<sub>50</sub>'s of both insecticides are plotted in Fig. 2 and isoboles suggests that the synergism increased with increase of simazine ratios. Since the synergistic actions of methomyl-simazine combinations are mainly due to the inhibition of biological oxidation. The mixtures of methomyl and PBO also showed similar pattern in synergism. In this investigation the same ratios were used as for simazine. At all ratios with cotoxicity coefficients more or less similar in each case (Table 2B).

In the study of synergistic combinations of methomyl and simazine, it was found that the toxicity to house flies can highly synergised. It was suggested by Sun and Johnson (1960) that the synergistic effect of sesamex and related compounds was due to the inhibition of biological oxidation. If we assume that the same mechanism of action occurs also to the combinations tested here. Oxidative detoxification in house flies may affect more types of compounds that we realize. Lichtenstein *et al.* (1973) found that the herbicide atrazine, simazine and monuron increased the toxicity of a range of insecticides to the house fly and among them atrazine was most effective increasing significantly ( $P = 0.1\%$  level) to the toxicity of parathion. The synergistic effect of simazine with permethrin were measured with the house fly by Wilkins and Khalequzzaman (1993) who observed increased synergism with increase

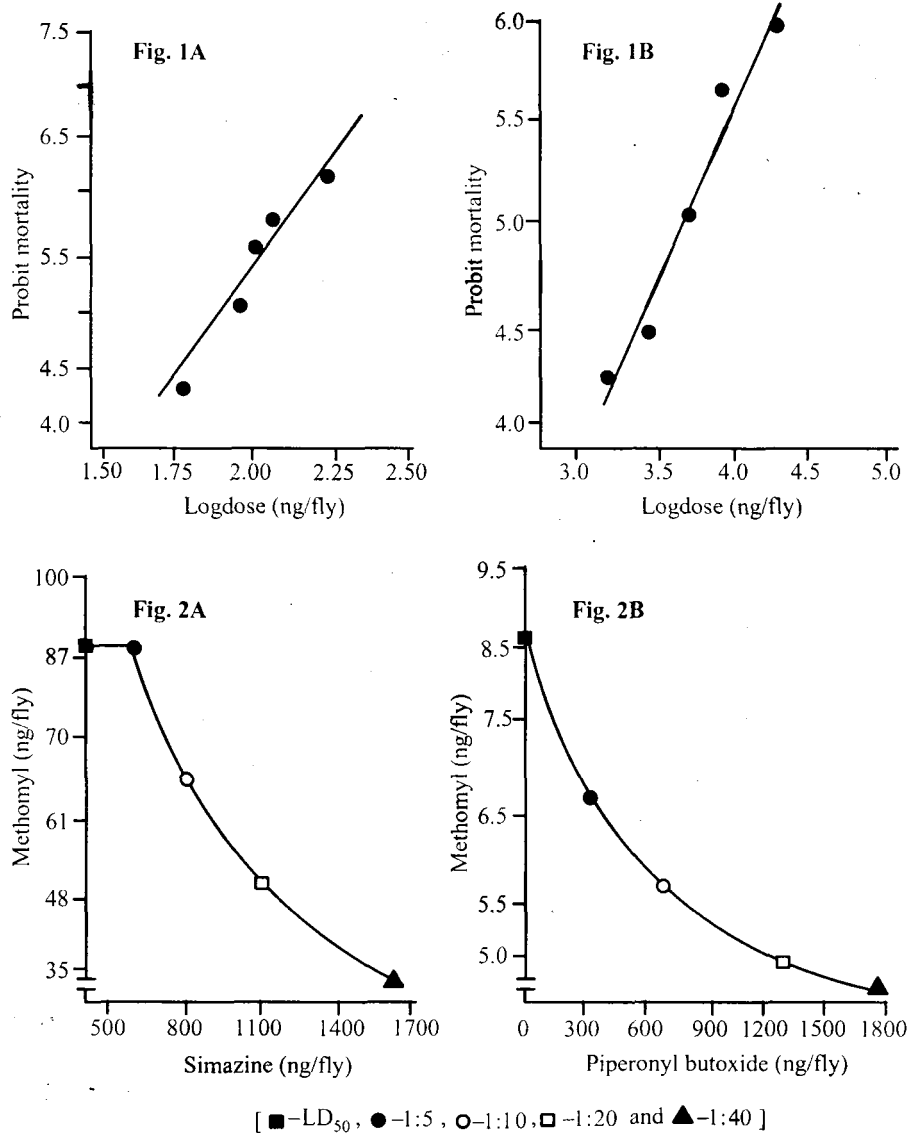


FIGURE 1. Regression lines of probit mortality on log dose of methomyl topically applied (A) and simazine topically applied (B).

FIGURE 2. Isobole of simazine and methomyl (A) and PBO and methomyl (B).

simazine ratios. The synergistic effect of atrazine with parathion in water or in soil were measured with mosquito (*Aedes aegypti*) by Liang and Litchenstein (1974) who observed that soil reduced the effectiveness of the insecticidal effect. Atrazine which is not toxic by itself, increased the toxicity of insecticide significantly. Litchenstein *et al.* (1979) conducted for the studies on the house fly to test the effect of atrazine on the toxicity of organophosphorus insecticides and found similar results to those observed with carbofuran and atrazine. Degradation in insect of carbofuran was inhibited in the presence of atrazine as observed by Chio and Sanborn (1977) who contended that compounds capable of blocking the microsomal electron flow can potentiated the toxicity of certain insecticides.

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## A New Glycerine Drop Trap Method to Sample Eriophyoid Mites

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**ABSTRACT:** Glycerine drop trap (GDT) method, a new technique is described to assess populations of eriophyoid mites under microscope. Its ease and efficiency is compared with that of the conventional counting template (CT) method. The GDT method was found 33 per cent more efficient than the CT method in sampling populations of the coconut eriophyoid mite, *Aceria guerreronis* Keifer.

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**KEYWORDS:** Eriophyid mites, sampling method.

### INTRODUCTION

Eriophyoid mites are so minute and numerous that their populations are difficult to count. They are not evenly distributed on all parts of their host plant as well (Perring *et al.*, 1996; Muraleedharan *et al.*, 1988). This calls for a better sampling technique so that population sampling is unbiased and the method simpler. Various methods have been proposed by different authors to count the mite numbers in a unit area. It was Youters and Miller (1934) who first made a 'counting template' (0.5" square) cut in a piece of paper to sample the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead). In another method an imprint of mites present on a leaf has been made by placing the leaf between two papers of proper absorptiveness and by crushing the paper against the mites to get a semi-permanent record of all stages of mite (Jeppson *et al.*, 1975). A combination of alcohol and ultrasonic vibration was used by Gibson (1975) who estimated the density of *Aculodes dubius* (Nalepa) on rye grass. Ramarethinam *et al.* (2000) used the same method to sample the coconut eriophyoid mite *Aceria guerreronis* Keifer. Zacharda *et al.* (1988) used an alcohol based technique without ultrasonic vibration as proposed by Gibson (1975) to monitor *Aculus schlechtendali* (Nalepa) in apple orchards. Allen (1976) estimated densities of *P. oleivora* on citrus fruit using a 10x hand lens mounted over a piece of clear plastic etched with a 1 cm<sup>2</sup>

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grid. The grid was divided into 25 equal subdivisions each having an area of  $4 \text{ mm}^2$ . Moore and Alexander (1987) assessed the populations of *A. guerreronis* by using a log scale in each tepal (0 = no mites, 1 = 1 to 10 mites, 2 = 11–100 mites), especially when infestations were very heavy. Gipsert *et al.* (1989) counted the tomato russet mite, *Aculops lycopersici* by placing the tomato leaflets between two glass microscopic slides with the slide on lower leaf surface etched with three  $1 \text{ cm}^2$  squares corresponding to medial, central and terminal leaflet.

We developed a simple technique, glycerine drop trap (GDT) method, to assess with the aid of a binocular zoom stereo microscope the population per unit area of *A. guerreronis* infesting the surface of tender coconut drupe/tepal. Usually, when viewed through the microscope, innumerable eriophyoid mites appear motile. Therefore it would be difficult to count them from a fixed unit area. On the other hand, when a drop of glycerine (Ar grade) is placed on the mites, the hemispherical viscous drop traps a fixed number of mites in that circular area (Plate ). These mites are easy to count as they are rather motionless. A narrow beam of illumination (e.g. a fibre optic cold light) from any one of the sides brings the trapped mites to clear view. The glycerine drop can be placed on the mite infested surface (perianth tepal or nut surface) using a steel pin-head of required size. A 24 mm long pin with  $1.7 \pm 0.15 \text{ mm}$  wide head was used in this study. The sharp end of the pin can be attached to a plastic handle with its pin-head in terminal position. When this pin-head was dipped in glycerine and taken out gently, the pin-head carries a glycerine drop that can be placed on the infested surface. When viewed through (eye piece 15x and objective 1x), the droplet covered a mean area of  $7.06 \pm 0.09 \text{ mm}^2$  ( $\#7 \text{ mm}^2$ ) assessed by finding its radius through micrometry. The mites trapped inside each drop could be seen clearly with a beam of cold light focused from a lateral side. Keeping the tepal inside the well of a cavity block/embryo cup will not allow the tepal or drupe to slip.

An experiment was conducted to compare the efficiency of the GDT method with that of the counting template (CT) method. Populations of *A. guerreronis* were assessed from a unit area by following both the techniques. In the GDT method the mite population was assessed from a  $7.06 \text{ mm}^2$  area. To cover rather the same area ( $7.02 \text{ mm}^2$ ) in CT method, a  $2.65 \text{ mm}^2$  window was cut on a white card. This template was placed over the tepal surface and the mite population was counted. On each tepal the two-way sampling was made dividing each tepal into three sections (two sides and one middle). All the six tepals of a drupe were used from 20 nuts and the mean number of mites from the 360 drops/templates was arrived at. Student's paired 't' test was used to compare the overall mean number of mites from  $7 \text{ mm}^2$  from each method.

Given a uniform field of observation, the GDT method proved easier, more accurate and more reliable than the counting template (CT) method to assess the mite population (Table 1). As the mites in the field of observation in the GDT method are trapped motionless, it was easier to count them, especially inside the lens-like glycerine drop. On the other hand, mites in the field of observation in CT method made the sampling more difficult as they kept moving in and out of the template, giving room for error. It is likely that mites could be missed from the field or counted repeatedly.

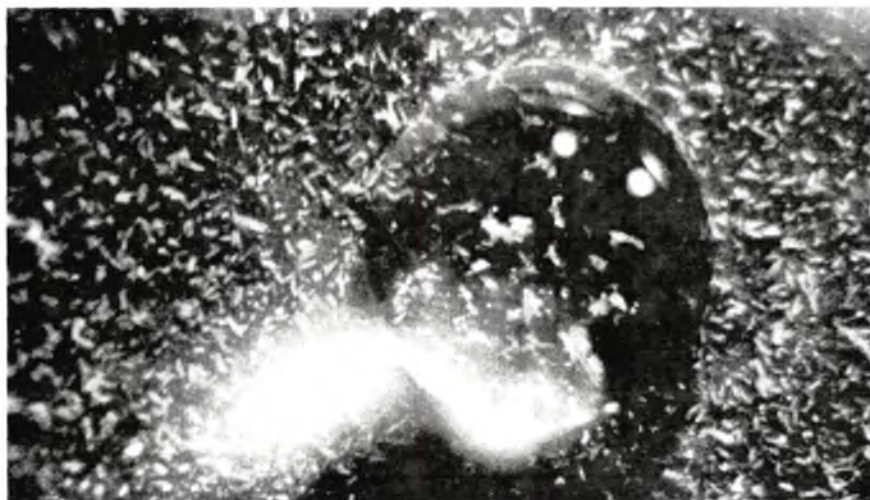


PLATE A hemispherical drop of glycerine placed on the tepal surface trapping the mites motionless in a rather circular area ( $7 \text{ mm}^2$ ). A narrow beam of light from any one of the sides brings the mites to clear view.

TABLE 1. Comparative efficiency of mite population sampling techniques

Sampling technique	No. of observations ( $n$ )	Mean number of mites counted/ $7 \text{ mm}^2$ area <sup>a</sup>
Glycerine drop trap (GDT) method	360	6.19 (2.59)
Counting template (CT) method	360	4.10 (2.13)

Figures in parenthesis are  $\sqrt{x + 0.5}$  transformed values.

<sup>a</sup>Significant difference ( $P < 0.05$ ), Student's  $t = 6.98$

Thus the GDT method was 33 per cent more accurate than the CT method since on average 2.09 more mites could be observed per  $7 \text{ mm}^2$  area in the former than in the latter. In the CT method (Youthers and Miller, 1934) active mites are quite difficult to count. Moore and Alexander (1987) used a log scale to assess the populations of *A. guerreronis*. On the other hand, the newly evolved glycerine drop trap (GDT) method is simpler, much easier and more efficient since it traps the moving mites in a fixed area easily selected at many places on the infested surface. Mites often tend to move in and out of the template which is comparatively difficult to keep track on the sampling surface. The GDT method permits both clarity and accuracy since the trapped but fresh mites can be leisurely examined in a fixed area.

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